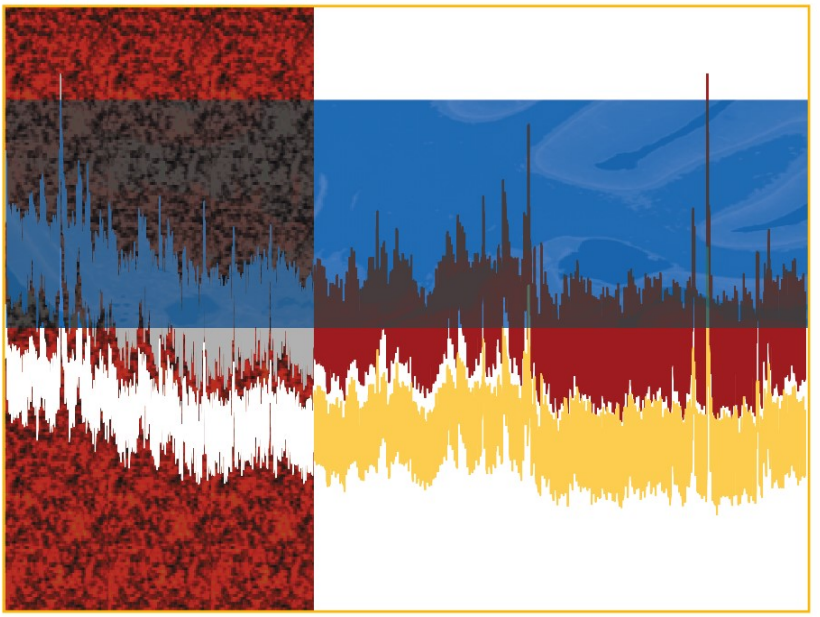

Cortico-Subcortical Dynamics in Parkinson's Disease

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Part I
Cortico-Subcortical Circuits and Parkinson's Disease

Chapter 1
Leading Toward a Unified Cortico-basal Ganglia Functional Model

Shannon R. Blume and Kuei Y. Tseng

Basal Ganglia Circuitry: The Direct and Indirect Pathways

There is a large body of literature establishing the basal ganglia functional organization of cortical afferents projecting to the striatum to two different pathways: the direct and indirect pathways [1–4]. Consistent throughout this organization are the medium spiny neurons (MSNs) which account for approximately 90–95% of the cell population in the striatum [5, 6]. MSNs are GABAergic projection neurons that are comprised by two functionally distinct groups of neurons based on the expression of neuropeptides and dopamine (DA) receptors [7]. More specifically, striatal neurons in the direct pathway contain substance P and dynorphin, and preferentially express D1-class DA receptors [8–10]. On the other hand, MSNs from the indirect pathway are enkephalin positive neurons and preferentially express D2-class DA receptors [8–10]. Despite that striatal neurons from the direct and indirect pathways exhibit opposite responses to DA, as D1 and D2 receptors are coupled to G_s and G_i, respectively, activation of these circuits typically result in inhibition of the basal ganglia output nuclei: the globus pallidus internalis (GPi) and the substantia nigra pars reticulata (SNpr) (Fig. 1.1A). For instance, striatal D1 activation will increase MSNs firing and thereby increase the inhibition of the basal ganglia output nuclei (GPi/SNpr), leading to disinhibition of the thalamocortical loop [11, 12]. Similarly, D2 activation will result in disinhibition of the globus pallidus externalis (GPe), increased inhibition of the subthalamic nucleus (STN), which in turn reduces the excitatory tone to the output nuclei and thalamocortical disinhibition. Thus, DA activation of MSNs in the direct and indirect pathways leads to a synergistic outcome in the basal ganglia output nuclei [13].

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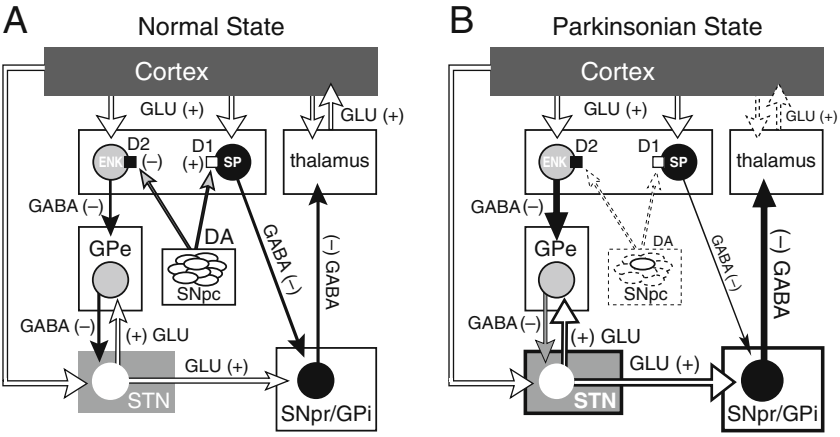

Fig. 1.1 Functional model of basal ganglia circuitry in normal (A) and parkinsonian (B) state. *White* and *black arrows* indicate excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmission, respectively. *Enlarged* and *broken arrows* in B indicate pathways that are believed to be upregulated and downregulated after chronic dopamine depletion, respectively

Functional Changes in the Basal Ganglia After Dopamine Depletion

When chronic DA depletion occurs in the basal ganglia, as seen in Parkinson's disease (PD), synaptic transmission within the direct and indirect pathways becomes imbalanced (Fig. 1.1B) [14, 15]. The lack of D2 tone in the indirect pathway will increase inhibition of the GPe and lead to disinhibition in the STN, thereby increasing the excitatory tone to the GPi/SNpr. The lack of D1 receptor activation in the direct pathway will result in less inhibition of the output nuclei. Thus, output nuclei hyperactivity in PD is expected as the functional consequence of two parallel processes: (i) a reduced activity of the inhibitory direct pathway and (ii) an increased activity of the STN. Increased STN activity is believed to result from disinhibition of striatopallidal neurons and the subsequent reduction in tonic inhibitory input from the GPe to the STN [3, 16]. In conjunction, excessive activation of the output nuclei would lead to a decrease in the excitatory thalamic output to the cortex, thereby causing the motor deficits commonly seen in PD (Fig. 1.1B). This pathophysiological model is supported by the fact that STN inactivation/lesion ameliorates motor deficits in PD and experimental parkinsonism [17–21] and by compelling evidence revealing compatible neurochemical and metabolic changes in animal models of parkinsonism: (i) unilateral nigrostriatal DA lesion induced by intracerebral injection of 6-hydroxydopamine (6-OHDA) in rats and (ii) DA depletion induced by systemic administration of methyl-phenyl-tetrahydropyridine (MPTP) in non-human primates [3, 16, 22, 23].

Glutamate Decarboxylase

Glutamate is the precursor for the synthesis of GABA by the rate-limiting enzyme glutamate decarboxylase 67 (GAD_{67}). Thus, GAD_{67} expression could be used to determine changes in activity of GABA-containing cells such as those in the basal ganglia (Fig. 1.1) [24]. For example, an upregulation of GAD_{67} mRNA in SNpr [25, 26] and GPi [24, 25, 27] was consistently observed in non-human primates treated with MPTP when compared to saline controls. More importantly, L-DOPA treatment reversed these changes [24, 26, 27]. On the other hand, GAD_{67} expression in the GPe and striatum has shown to be less clear. Herrero and colleagues found no significant changes in GAD_{67} mRNA expression in the GPe of MPTP-treated animals, whereas Soghomonian's group found a significant increase [25, 27].

Similar to what was found in animals treated with MPTP, 6-OHDA-lesioned rats also showed an increase in GAD_{67} expression in the basal ganglia output nuclei [28–30]. However, GAD_{67} mRNA expression is also increased in the striatum of DA-depleted rats, a finding that is fairly consistent across different research groups [28–31] Again, all the changes induced by 6-OHDA were normalized after L-DOPA treatment [28].

Overall, the main findings remain as predicted, that is, neuronal activity in the GPi and SNpr is increased in DA-depleted animals, an effect that can be restored to near normal levels with L-DOPA treatment.

Cytochrome Oxidase

Among the diverse metabolic activities required to maintain neuronal function, the most important ion pump is the Na^+/K^+-ATPase, which consumes ~60% of brain ATP [32, 33]. Interestingly, the energy (ATP) supply is almost completely derived from the oxidative metabolism of glucose [33], whose final stage is catalyzed by the cytochrome oxidase I (CO-I). CO-I is the terminal enzyme of the mitochondrial electron-transport chain that provides most of the ATP used in the brain [34] and is a useful marker of brain metabolic activity. This enzyme, also known as "complex IV" of the mitochondrial chain, is composed of 13 subunits, 3 of which are encoded by the mitochondrial genome and 10 by the nuclear genome [35]. Wong-Riley and co-workers have showed that a mono-ocular injection of tetrodotoxin, a procedure used to inhibit neuronal activity in the geniculate body, also induced a profound decrease in local CO-I activity [36]. Both CO-I protein levels and mRNA coding for the subunits of CO-I are responsive to changes in neuronal activity allowing analyses of regional, cellular and subcellular functional levels [34, 37, 38].

Metabolic measures using CO-I in DA-depleted animals supported the data obtained with GAD_{67}. Both CO-I histochemistry and mRNA are increased in the GPi, STN and SNpr in non-human primates exposed to MPTP when

compared to controls [28, 39, 40]. L-DOPA treatment reverses the changes induced by MPTP. Similarly changes in CO-I levels were observed after chronic DA depletion in rats. For instance, 6-OHDA-lesioned animals demonstrated increased CO-I levels in GPi and SNpr [41], as well as the STN [42]. These changes in CO-I expression were correlated with changes in neuronal firing pattern [22, 42]. It is also important to note the lack of consistent changes in the GPe regarding CO-I levels and GAD_{67} expression [27] after DA depletion suggests that the hyperactive STN state in PD may not be exclusively mediated by a disruption of the indirect pathway/GPe.

2-Deoxyglucose

In 1977, Sokoloff and colleagues developed an autoradiographic 2-deoxyglucose (2-DG) method for measuring glucose utilization in the various structures of the brain in both normal and experimental conditions [43]. Compared to CO-I histochemistry, 2-DG reflects better neuronal activity occurring over shorter time periods lasting from seconds to minutes, rather than hours or weeks. Although the anatomical resolution of CO-I histochemistry is better than that of 2-DG autoradiography [34, 44], changes in 2-DG uptake have been experimentally found to reflect mostly changes in synaptic, not cellular, activity. For instance, Kadekaro and collaborators found that 2-DG in the dorsal root ganglia is taken up mostly by the axon terminals [45].

Numerous studies have investigated the impact of DA lesion on the functional anatomy of the basal ganglia circuitry [46–50]. Consistent throughout the literature, glucose uptake is decreased in the STN and increased in the GPe after DA depletion, yet no significant changes were found in the GPi and striatum [47–50]. Despite the differences between CO-I and 2-DG studies, the main conclusions remain as predicted, in particular regarding changes in the indirect pathway: (i) glucose uptake is increased in the GPe resulting from the amplified striatal GABAergic output due to the lack of D2 receptor activation and (ii) glucose utilization is decreased in the STN from the lack of inhibitory input from the GPe.

Chronic Dopamine Depletion and Basal Ganglia Oscillations

In vivo electrophysiological studies have uncovered other aspects of the basal ganglia functional organization that cannot be easily conciliated with the classic model described above. For instance, the predicted hyperactivity of output nuclei neurons, commonly measured as an increase of the mean firing rate, was reported by some groups [51–53], but not by others [54–62]. Interestingly, changes in firing pattern have consistently been reported in recent studies: neurons in the output nuclei tend to fire in bursts of action potentials and

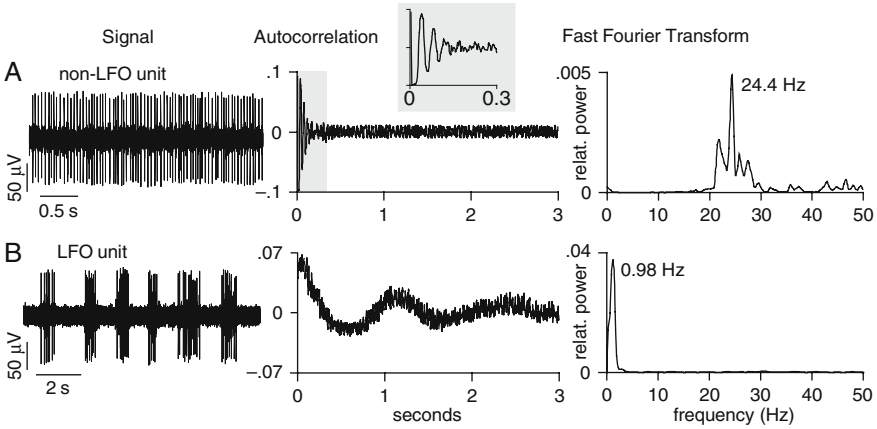

Fig. 1.2 Substantia nigra pars (SNpr) reticulata single-unit recordings in control and 6-OHDA-lesioned rats. Neurons in the SNpr can be classified into two main categories based on the autocorrelograms (*middle panel*) and the power spectra (*right panel*) of the interspike intervals. (**A**) The majority of cells recorded from control animals exhibit tonic/regular firing patterns (non-LFO) and display dominant peak within 20–30 Hz. (**B**) After chronic dopamine depletion, ~40% of neurons in the SNpr show rhythmic burst firing activity (LFO) with a dominant peak frequency of ~1 Hz. Modified from Tseng et al [66]

show periodic oscillations in firing rate (Fig. 1.2). Unfortunately, only firing rate, not firing pattern has been considered to drive the relationship between the direct and the indirect pathways (Fig. 1.1). In the following section we will (i) review evidence regarding the role of the striatum and the STN in the regulation of oscillatory activity in the basal ganglia and (ii) summarize recent data on how chronic DA lesion of the nigrostriatal pathway impacts the cortico-basal ganglia dynamics. We will conclude with an integrated/unified functional cortico-basal ganglia-thalamocortical model by taking into account the temporal domain of neuronal oscillations from single cell to the system level.

Chronic Dopamine Depletion and Firing Pattern Shift in the Basal Ganglia

Basal ganglia output neurons in normal and control rats typically display regular firing patterns ranging from 15 to 40 Hz when recorded in vivo under general anesthesia [13, 54, 56, 57, 60, 63–69]. After chronic DA depletion (i.e., 6-OHDA rats), 40–50% of SNpr neurons fire bursts of action potentials and display low frequency oscillatory (LFO) activity (~1 Hz) [57, 64, 66–70] (Fig. 1.2). STN lesion significantly decreased burst firing in the SNpr of 6-OHDA-lesioned rats, suggesting that the STN could mediate the emergence

of abnormal oscillatory activity after chronic DA depletion. In fact, neurons of the GPe and STN display synchronized oscillatory burst discharge at LFO (0.4–1.8 Hz) in mature organotypic cultures containing striatal and cortical tissues, but lacking DA neurons [71]. Similarly, oscillatory burst discharge can be induced by sustained membrane hyperpolarization in vitro in slices containing STN neurons [72]. The role of the STN in mediating oscillatory burst firing in the basal ganglia is further supported from a recent work by Magill and co-workers demonstrating that STN and GPe neurons discharge LFO bursts in vivo in ketamine-anesthetized rats [73]. Interestingly, LFO activity within the GPe-STN network is correlated with changes in cortical field potential, suggesting that slow cortical rhythms are propagated via a cortico-STN-GP network.

Recent studies indicate that chronic DA depletion enhances LFO in the STN and output nuclei neurons. STN neurons recorded from urethane-anesthetized 6-OHDA-lesioned rats display a significantly higher number of cortically driven LFO burst units (Fig. 1.3) [64, 69, 74, 75]. Similarly, periodic burst firing in the SNpr of 6-OHDA-lesioned rats is correlated with slow cortical rhythms [66, 70]. It can be speculated that slow cortical rhythms are transferred to the SNpr via the STN in the parkinsonian state as STN lesion reduced burst firing in the SNpr [57, 67, 68]. However, Plenz and Kitai also reported that neurons in the GPe and STN can sustain phase-locked synchronized LFO, even in the

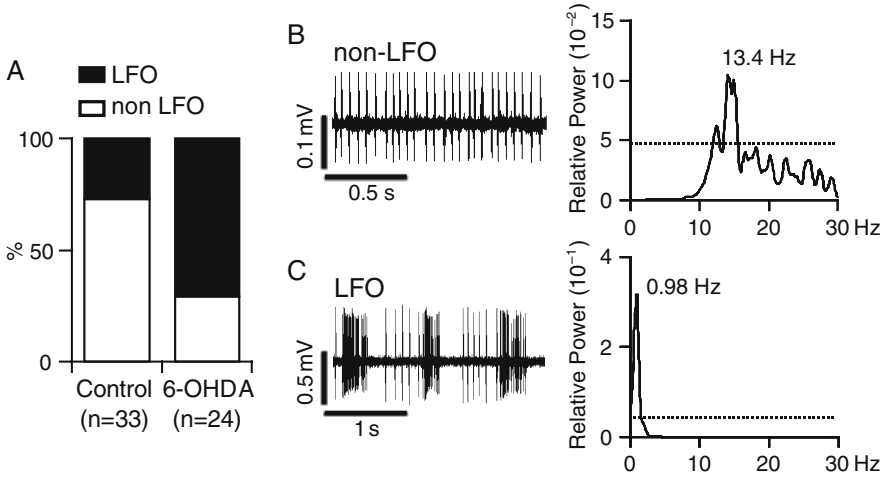

Fig. 1.3 Single-unit recordings of subthalamic nucleus (STN) cells in control and 6-OHDA-lesioned rats. (**A**) Cells showing burst firing activity (i.e., LFO units) were found in both groups though to a greater extent in animals with chronic dopamine depletion (i.e., 6-OHDA) (9/33 vs. 17/24, normal vs. 6-OHDA LFO units, respectively; $p = 0.0016$, Fisher exact probability test). Thus, non-LFO units predominated in control animals. (**B**) Non-LFO units displayed dominant frequency peaks within 10–30 Hz as revealed by the power spectra of the interspike interval. In contrast, LFO units display rhythmic burst of action potentials with a dominant peak frequency within 1 Hz

absence of cortical inputs, in organotypic cultures lacking DA neurons [71]. Consequently, the possibility that both the cortex and the GPe–STN networks contribute equally to the generation of LFO-bursting activity in the parkinsonian basal ganglia cannot be completely ruled out, yet they are not mutually exclusive.

There is also evidence indicating that a disruption of striatal function could underlie the appearance of LFO activity in the basal ganglia after chronic DA depletion [57, 68]. For example, stimulation of striatal D1-class DA receptors changes the firing pattern of SNpr neurons of 6-OHDA-lesioned rats, from a LFO to a non-LFO firing mode [68]. D2 activation, however, had little impact on SNpr LFO firing [68], yet a robust effect on the mean firing rate was observed [68]. These findings suggest divergent roles for striatal D1 and D2 receptors in the modulation of output nuclei firing pattern, an effect that may be related to how DA receptors regulate striatal neurons function and corticostriatal transmission [76].

Corticostriatal Function and Basal Ganglia Oscillations

As discussed above, inputs to the striatum arise from the cerebral cortex, thalamus and midbrain. At the cellular level, both DA-containing fibers and cortical glutamatergic inputs extensively converge and interact in the same striatal projection cells, known as MSNs. These neurons exhibit membrane potential fluctuations when recorded in vivo (Fig. 1.4). A more hyperpolarized level, also known as down state, is interrupted by periods of sustained depolarization, the up states [77]. The onset of these events requires strong excitatory synaptic inputs driven from the cerebral cortex and thalamus [77, 78] to overcome the tight hold the inwardly rectifying K^+ current provides to the membrane potential during down states. Yet there is still an ongoing debate regarding whether continuous glutamatergic inputs are needed to sustain the depolarization [79], it becomes clear that neuromodulators such as DA can contribute to sustain plateau depolarizations by activating/inactivating intrinsic voltage-gated channels and by modulating the strength of local glutamatergic excitation [80]. Thus, plateau depolarizations or up states can be perceived as "enabling states", during which synchronous corticothalamic activity is translated into sequences of action potentials in MSNs, allowing transmission of processed information to the output nuclei.

It has been hypothesized that chronic DA depletion may facilitate the transmission and expression of thalamocortical oscillations in the basal ganglia as LFO burst firing [81] and that stimulation of striatal D1-class DA receptors prevents the spreading of the rhythm [68, 82]. It remains to be determined whether the effect of striatal D1 stimulation on output nuclei LFO activity is mediated by the direct pathway (i.e., striatonigral projection) itself or by modulating the GPe–STN network oscillations, two plausible mechanisms that are not mutually exclusive.

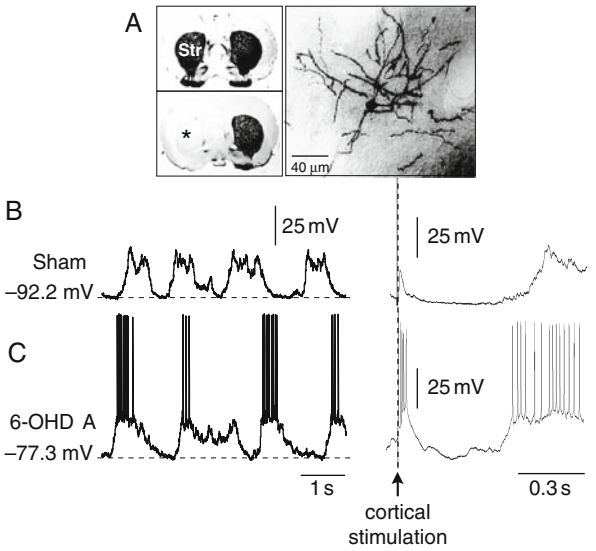

Fig. 1.4 In vivo intracellular recordings of striatal output neurons (i.e., MSN) in sham-lesioned and 6-OHDA-lesioned rats. (**A**) Coronal sections immunolabeled against tyrosine hydroxylase show representative tissue from sham and 6-OHDA-lesioned animals. Neurobiotin staining confirms typical morphology of striatal MSN cells. (**B** and **C**) Striatal neurons from control and 6-OHDA-lesioned animals displayed fluctuating membrane potentials, from a hyperpolarizing DOWN state to a depolarizing UP state. Compared to the sham group, striatal neurons from 6-OHDA-lesioned animals display a more depolarized membrane potential, an increase in probability of firing during the UP states. Thus, MSN in 6-OHDA animals are more responsive to cortical stimulation as revealed by the shorter and the more depolarized membrane potential during the long-lasting hyperpolarizing–depolarizing phase that typically follows after the monosynaptic postsynaptic potential

Cortical neurons also exhibit periodic oscillations in their membrane potentials that are strongly correlated with the EEG oscillatory activity and local field potential when recorded in vivo [83]. Only during the depolarizing phase do cortical neurons fire action potentials, usually as bursts of spikes, which in turn would allow transmission of the corticothalamic rhythms [83]. Interestingly, striatal MSN membrane potential fluctuations appear to reflect spreading activity of cortical field potential oscillations [81]. This conclusion is supported by previous findings indicating that MSNs up states are driven by inputs from the cerebral cortex and thalamus [77, 78, 84–86]. Thus, spreading of cortical rhythms to striatal target nuclei seems to be constrained by the very low firing probability of MSNs in healthy animals (Fig. 1.4B). In contrast, striatal MSNs recorded from rats with chronic DA depletion displayed a more depolarized membrane potential (both during the *down* and the *up* state), exhibited a significant increase in the probability of firing during the up states and are more responsive to cortical stimulation (Fig. 1.4C). Accordingly, neurons in the GPe and SNpr display tonic regular firing during natural slow wave sleep [63, 87] and

anesthesia [56, 57, 88, 89]. Following chronic nigrostriatal lesions, neurons in the basal ganglia display rhythmic burst firing, which is correlated to cortical slow wave activity [66, 70, 90, 91] (Fig. 1.2) and is strongly modulated by striatal DA receptors [57, 68].

Altogether, these findings suggest that more excitable striatal MSNs can be driven easily to the depolarized state and facilitate transmission of cortical rhythms to striatal target nuclei [81]. The mechanisms leading to these changes remain to be determined. Current knowledge suggests that both pre- and post-synaptic mechanisms account for the increased impact of cortical inputs on striatal activity after chronic DA depletion [92–94].

Dopamine-Dependent Regulation of Cortically Driven Oscillatory Activity in the Basal Ganglia and Akinesia

As described above, chronic nigrostriatal lesion increases the proportion of basal ganglia neurons showing rhythmic firing rate modulations coupled to cortical oscillations [67, 70, 74]. Similar coupling between cortical activity (i.e., EEG and cortical field potential) at the STN and GPe has been reported in awake PD patients [95] and behaving rats with DA lesion [96]. Thus, it appears that the firing pattern of an important population of neurons in the basal ganglia becomes locked to cortical activity, an effect that may alter the coordination and selection of afferent signals required to establish specific task-directed behaviors [67, 97]. Consequently, an enhancement of this cortically dependent oscillatory activity could be associated to the emergence of motor deficits in PD such as tremor, akinesia and rigidity [67, 97]. This is supported by the fact that inactivation of the STN alleviates PD symptoms [18, 19, 98–100] and reduces the proportion of LFO units induced by chronic DA depletion in the basal ganglia [57, 68, 81].

DA receptor activation within the cortico-basal ganglia network also reduces the exaggerated cortically dependent oscillatory activity induced by chronic DA depletion [13, 68, 82, 96]. Therefore, it is possible that the emergence of cortically driven oscillatory activity in the basal ganglia occurs when the level of DA depletion is >70%, that is, when motor impairments in PD becomes clinically evident. By examining the electrophysiological changes in the output nuclei of animals with different degrees of DA cell loss, we found that this was the case. Cortically driven bursting activity in the basal ganglia becomes evident in animals with ~70% of mesencephalic DA denervation (~75% in the SN and ~55% in the ventral tegmental area [VTA]), but not in those with ~60% of DA lesion in the SNpc with an intact VTA [66] (Fig. 1.5). More importantly, animals with >95 and ~70% DA cell loss exhibited similar stepping test deficits and electrophysiological changes. A similar relationship was also observed between the level of DA lesion and the appearance of stepping deficits, a behavior test used to evaluate akinesia in rats [101–103].

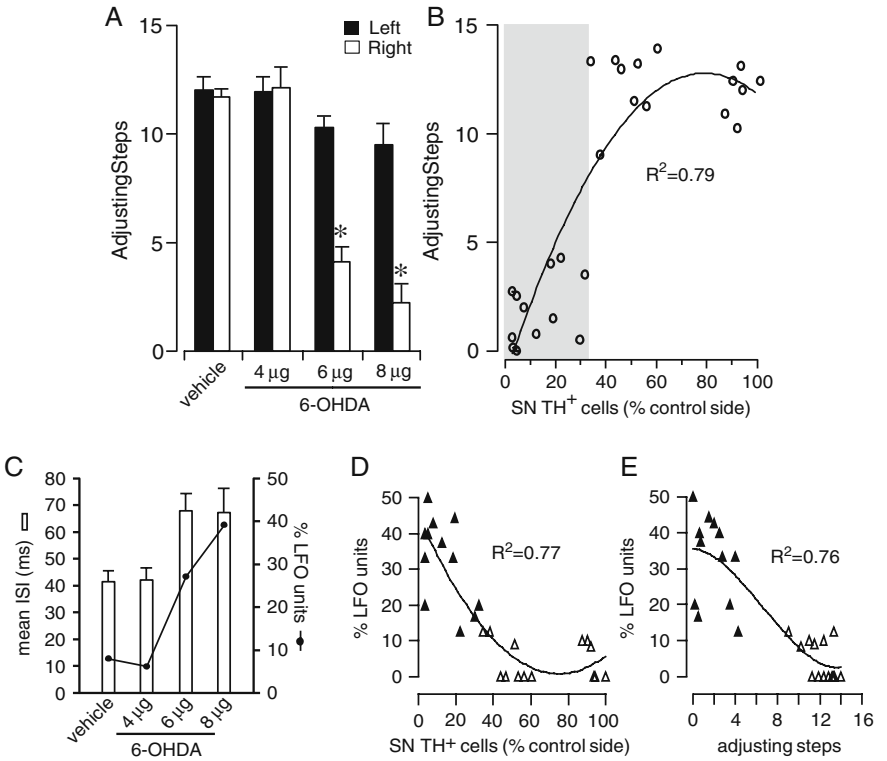

Fig. 1.5 Linking motor deficits, the degree of dopamine lesion and appearance of the abnormal oscillations in the basal ganglia. (A) Stepping test deficits were not apparent in vehicle or 4 µg 6-OHDA-lesioned groups, but were evident in 6 and 8 µg 6-OHDA-lesioned groups. (B) The relationship between SN TH^+ cell depletion and stepping performance (in the contralateral limb) is demonstrated in this scatter plot. The shaded region shows the animals from the 6 and 8 µg 6-OHDA-lesioned animals. (C) Double *y*-axis plot summarizing the relationship between the mean interspike interval (ISI; left *y*-axis) and the proportion of LFO units (right *y*-axis). Different degrees of dopamine denervation can be obtained increasing doses of 6-OHDA. Animals in the 6 and 8 µg 6-OHDA-lesioned groups demonstrated a similar increase in proportion of LFO units and mean ISI, whereas animals in the 4 µg 6-OHDA-lesioned groups demonstrated similar proportions of LFO units and mean ISI to the vehicle group. (D & E) Scatter plots illustrate the relationship between the percentage of LFO units in relation to the level of dopamine denervation (i.e., number of TH^+ cells in the SN) and the stepping test performance (i.e., number of stepping adjustment). Open triangles represent animals from vehicle and 4 µg 6-OHDA-lesioned groups. Solid triangles correspond to animals that received 6 and 8 µg of 6-OHDA. (D) Animals with the greatest TH^+ cell loss showed a greater proportion of LFO units. (E) Similarly, animals with deficits in stepping performance showed a greater proportion of LFO units. The solid lines indicate the second-order polynomial (D) and sigmoidal (E) (Boltzmann model) regression best fitted for all data points, respectively. Modified from Tseng et al [66]

These results indicate that the presence of cortically dependent oscillatory firing pattern in the basal ganglia after chronic DA depletion could be an important pathophysiological feature of the parkinsonian state [66]. Thus, an appropriate level of DA signal is required to maintain the proper temporal coupling and translation of afferent activity between the thalamocortical system and the basal ganglia nuclei.

Integration of the Motor-Limbic Circuits in the Basal Ganglia

One of the major roles of the basal ganglia is to integrate sensorimotor, associative and limbic information in the production of context-dependent behaviors [104–107]. Traditionally, the *dorsal* division of the basal ganglia (dorsal striatum [ST], GP, STN and substantia nigra) is implicated in sensorimotor control, whereas the *ventral* division (ventral striatum or nucleus accumbens [NAc], ventral pallidum [VP] and ventral tegmental area) is associated to limbic/cognitive functions. It is well known that the cerebral cortex and the basal ganglia are functionally related via a multisynaptic loop [108]. As discussed above, the information carried by the corticostriatal pathway is processed and integrated in the striatum and transmitted to the output nuclei via the *direct pathway* or through the complex network interconnecting the GPe and STN, the *indirect pathway*. Despite the corticostriatal system, growing evidence indicates that a direct *cortico-subthalamic pathway* could convey similar cortical information to the basal ganglia [65, 73, 87, 109–113]. Interestingly, the STN functions are not limited to motor coordination and movement control. Whereas the lateral part of the STN receives projections from motor cortical areas, connections of the medial STN with the prefrontal cortex and the limbic-associated regions of the basal ganglia [55, 114–117] emphasize that the STN also contributes to non-motor behavior [118, 119]. In a series of very elegant experiments, Baunez and co-workers demonstrated that the STN plays a critical role in the regulation of impulsive actions as indicated by specific deficits in reaction time tasks in animals with STN lesion [118–120]. Indeed, the non-motor functions of the STN could reflect its reciprocal projections with the VP (Fig. 1.6), which receive corticolimbic information via the NAc [108, 117, 121, 122]. In addition, a role of STN in spreading cortical rhythms to the basal ganglia is also supported by two recent reports showing that (i) the proportion of rhythmic bursting neurons in the GPe is almost abolished after STN lesions in 6-OHDA-lesioned rats [88] and (ii) the change in the STN activity following 6-OHDA-lesions and cortical ablation is not associated with changes in GP neurons activity [74]. Taken together, it is tempting to speculate that the STN in conjunction with the dorsal and ventral striatum provides the two main pathways through which cortical information is integrated in the basal ganglia.

In non-human primates DA depletion also shifted the firing pattern of basal ganglia neurons from a non-oscillatory mode to a rhythmic burst firing [58, 59,

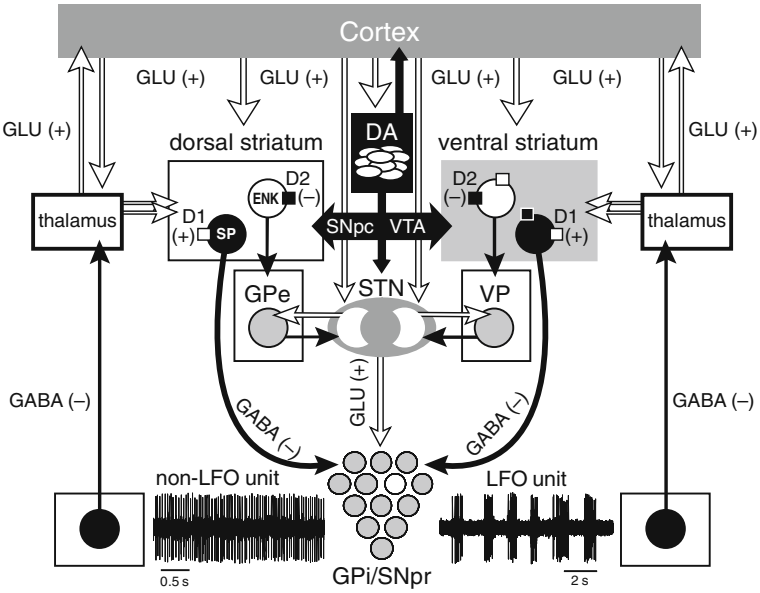

Fig. 1.6 An integrated functional cortico-basal ganglia–thalamocortical model that includes neural circuits from motor and limbic structures. In the classic parkinsonian model of basal ganglia circuitry (Fig. 1B) the effects of dopamine depletion is primary focus on the dorsal striatum. However, dopamine depletion in PD takes place in both the SNpc and the VTA, changing both the dorsal and the ventral striatal circuits as well as the mesocortical regions. In addition, the subthalamic nucleus (STN) may play a critical role in mediating the integration of information from sensorimotor and limbic-/cognitive-related regions of the cortex to the dorsal and ventral striata via reciprocal projection with the globus pallidus (GPe) and ventral pallidum (VP), respectively

62, 123]. Similarly, a substantial proportion of neurons in the STN and GPe exhibit oscillatory firing in patients with PD [124–127]. Despite that it has been proposed that such oscillatory activity gives rise to tremor, it has been noticed in the absence of tremor as well. Since bursting activity has been suggested to play a specific role in synaptic plasticity and information processing [128], it seems likely that the emergence of abnormal oscillatory activity in the basal ganglia would result in inappropriate integration of excitatory/inhibitory inputs and disruption of cortical information processing by promoting abnormal synchronous activity within the thalamocortical network [129]. More importantly, impairments in executive functions such as working memory and attention deficits as well as difficulties in initiating goal-directed behaviors have been frequently observed in PD [130, 131]. Although L-DOPA therapy in early PD improves motor symptoms, its effects on cognitive performance are more complex and controversial [132]. Both positive and negative effects have been observed, suggesting that both motor and cognitive deficits in PD may or may not share a common neuropathophysiological substrate [130, 131]. Thus,

DA-depletion-induced oscillatory activity may have a more general impact in the genesis of the parkinsonian state that includes both motor impairments and cognitive deficits.

Summary and Conclusions

Although the appearance of abnormal oscillatory activity observed in PD resembles what happens in rats after 6-OHDA-induced lesions, the main frequency of oscillatory activity in behaving animals (3–20 Hz) is higher than that found in anesthetized 6-OHDA-lesioned rats (0.4–2 Hz) [67, 81]. If abnormal spreading of cortical rhythms through the striatum and the STN underlies the rhythmic firing pattern of output nuclei neurons in PD, the different oscillatory activity observed in awake parkinsonian primates and anesthetized 6-OHDA-lesioned rats may merely reflect the distinct dominant cortical frequencies which characterize each behavioral state.

In summary, DA plays a critical role in regulating the flow of cortical information to the basal ganglia network. In PD, chronic DA depletion profoundly alters the firing pattern of basal ganglia neurons and induces aberrant signal coding of cortical information from the striatum and STN to the output nuclei. Because neurons in the basal ganglia receive inputs from multiple cortical and subcortical regions with different functions, and their outputs also target cortical areas involved in cognition, the sensorimotor and cognitive deficits observed in PD and animal models of parkinsonism could simply reflect alterations in the integration and processing of cortical information [104].

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Chapter 2
Modeling Parkinson's Disease: 50 Years Later

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A Turning Point in the History of Neuroscience: *The Discovery of L-Dopa Decarboxylase*

The aromatic amino acid L-3,4-dihydroxyphenylalamine (also known as L-dopa or levodopa) was first synthesized by Casimir Funk (Berne, Switzerland) in 1911 and 2 years later, it was isolated from legumes (*Vicia faba*) by Marcus Guggenheim (a Hoffmann-La Roche biochemist, 1913). However, it was not until the discovery of the enzyme "L-dopa decarboxylase" by Peter Holtz in Germany in 1938 that the formulation of the "catecholamine pathway" could be introduced by Hermann Blaschko (Physiological Laboratory at Cambridge, UK) in 1939: L-tyrosine → L-dopa → dopamine (DA) → noradrenaline → adrenaline. A new direction in the research of catecholamine pharmacology was initiated by Hotlz's experiment in mammalian kidney homogenates showing that a decarboxylation of L-dopa yields to the formation of 3,4-dihydroxyphenylethylamine, a biologically active amine also known as "dopamine", a short version of the full chemical name introduced by Henry Dale years later in 1952.

A decade after the discovery of the L-dopa decarboxylase, Wilhelm Raab [1] from Vienna, Austria, found a catecholamine-like substance (adrenaline) in brain samples from different species including humans. Raab also pioneered the study on the effect of systemic administration of L-dopa on brain catecholamine levels. Six years later, Kathleen Montagu and Hans Weil-Malherbe (Runwell Hospital in Wickford, UK) demonstrated for the first time that the L-dopa → DA transformation does occur in brain homogenates revealing the presence of the enzyme L-dopa decarboxylase in the brain [2, 3]. Six months later (February 1958), Arvid Carlsson (Lund, Sweden) confirmed the presence of DA in brain tissue and that L-dopa administration in reserpine-treated rabbits (a pharmacological manipulation that reduces catecholamine levels and induces a state of

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immobilization) not only fully restored mobility and wakefulness [4] but also restored the brain's DA and noradrenaline to normal levels [5]. A profound effect of L-dopa on both behavioral and electrophysiological outcomes was reported under several different experimental conditions between 1957 and 1960 [4, 6, 7].

Inspired by Raab and Gigee's [1] work, Bertler [8] from Carlsson's laboratory at Lund in Sweden (1959) and Sano [9] from Osaka in Japan demonstrated for the first time that the main source of DA in the brain is from the "corpus striatum". The striatum is one of the major brain structures of the basal ganglia known to be involved in the control of motor behaviors. It became clear for the first time that striatal DA could play a crucial role in regulating the central motor program and thus be responsible for the parkinsonism-like effects observed in reserpine-treated animals. However, the most significant finding associating brain DA levels and parkinsonism did not come to light until Ehringer and Hornykiewicz [10] provided the first postmortem study measuring and comparing DA and noradrenaline concentrations in brains from control subjects and patients diagnosed with Parkinson's disease (PD). A severe DA reduction in the caudate and putamen (corpus striatum in primates) was found in PD patients but not in brains obtained from individuals who suffered from other types of extrapyramidal syndromes. Soon thereafter, Birkmayer (neurologist) and Hornykiewicz conducted a trial in a group of 20 patients with Parkinson's disease and found that i.v. administration of L-dopa significantly improved all motor deficits, associated with akinesia, for several hours [11]. At the same time, a team led by Theodore Sourkes (a biochemist interested in L-dopa/DA metabolism and L-dopa decarboxylase inhibitors; Montreal, Canada) observed similar improvements in PD patients receiving oral L-dopa [12]. Six years later, another significant breakthrough for L-dopa as a therapeutic agent for PD emerged when George Cotzias in New York introduced, for the first time, the so-called high-dose oral L-dopa regimen [13]. At this time, Melvin Yahr (also from New York) initiated the first double-blind L-dopa trial. The double-blind study led by Yahr was concluded in 1969 with great therapeutic success by showing a clear superior effectiveness of L-dopa as an anti-PD agent [14].

Modeling Parkinson's Disease: *From DA Depletion to Genetic Manipulations*

Parkinson's disease (PD) is a progressive, neurodegenerative disorder of aging characterized primarily by motor symptoms such as tremor, rigidity and bradykinesia. The primary neuropathological feature of PD is the profound loss of DA nigrostriatal neurons, but abnormal reductions also occur in other DA and non-DA cells, and these appear either before or subsequent to the substantia nigra (SN) loss [15]. Importantly, clinical manifestations in PD do not emerge until the progressive damage of the DA system reaches a critical level, i.e., ~70–80% reduction in striatal DA terminals and ~50–60% loss of DA neurons in the SN [16]. It has been proposed that the delay in the appearance of

motor deficits with DA depletion is due to compensatory neuroadaptative mechanisms that normally occur at pre- and postsynaptic levels after a DA lesion [17]. Aside from neuron loss, other pathological signs include the accumulation of insoluble proteins, in particular, alpha-synuclein in the form of inclusions called Lewy bodies. These protein aggregates are found in the remaining nigral as well as cortical neurons, when human brains are assayed postmortem [18]. We know little of why these aggregates form or whether they contribute to DA neuron demise.

Patients with PD also exhibit cognitive deficits, even in the earliest stages [19]. In addition to an increased risk for clinical dementia and depression [20], PD subjects also exhibit cognitive difficulties resembling those observed in patients with frontal lobe damage and which mainly include the so-called frontal/executive deficits [21–30]. Impairments in executive functions such as working memory and attention deficits as well as difficulties in initiating goal-directed behaviors have been frequently observed in PD subjects [19]. Therefore, the evidence for reductions in cell number in the mesocortical pathway, which originates with the DA neurons in the ventral tegmental area and the medial SN [31], is of particular importance because it could help explain cognitive impairments in PD [32, 33]. Although early PD is characterized by motor symptoms that generally respond well to L-dopa therapy, changes in cognitive performance are more complex and even controversial [34]. L-dopa therapy can, in some cases, improve cognitive symptoms, suggesting that both motor and cognitive deficits share a common neuropathophysiological substrate [19].

Investigators rely heavily on rodent models of PD to gain insights into the cause, progression and symptomatic relief from the disease. Many different rodent models have been created, which mimic some aspects of the pathophysiology of the disease. The DA cell loss from the SN and the striatal DA depletion are more rapid with some protocols than with others, so replicating disease progression has not been easy.

Among the most widely used models are those that employ toxins, such as 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone or paraquat. Depending on the protocol used, these toxins show varied amounts of DA cell loss, striatal DA loss and behavioral deficits in rodents [35, 36]. More recently, inflammation-based models have been created. Lipopolysaccharide (LPS), an endotoxin derived from gram-negative bacteria, is a potent inflammatory toxin and is also used to model neuroinflammation in PD [37–39].

Unilateral DA Depletion Induced by Injecting 6-OHDA into the Brain

The neurotoxin, 6-OHDA, is structurally similar to DA and norepinephrine and has a high affinity for the plasma membrane transporters of these

catecholamines [40]. 6-OHDA is readily oxidized in the cell and produces hydrogen peroxide and paraquinone, both highly toxic [41]. This toxin is administered directly into the brain, where it specifically kills DA and noradrenergic neurons and their terminals [42, 43]. Injections are carried out under surgical conditions and the toxin is generally delivered unilaterally to the SN, medial forebrain bundle (MFB) or striatum. High concentrations destroy the SN DA cells first, within a few hours, before the striatal terminals [43]. Nevertheless, when 6-OHDA is injected into the MFB, striatal terminals degenerate first, followed by death of the SN neurons [44]. More importantly, the degree of DA lesion increases in both the SN and the ventral tegmental area in a dose-dependent manner [45]. When high concentrations of 6-OHDA are administered into the SN or MFB, striatal DA levels rapidly decrease by 90% concurrent to a nearly complete destruction of SN neurons and striatal terminals [44, 46, 47]. Sprouting of remaining terminals can follow the injury [48]. When 6-OHDA is injected in the striatum, the loss of DA nigrostriatal pathway is dose dependent and more progressive than when the toxin is introduced into the SN or MFB [49]. Interestingly, Stanic and colleagues [50] found that 16 weeks after a partial 6-OHDA unilateral lesion of the SN, the striatum is fully re-innervated, and nigral DA neurons recover completely by 32 weeks post-lesion. Fleming and colleagues [51] gave ascending doses of 6-OHDA to the striatum through a unilateral indwelling cannula over 14 days. They were able to induce a 35% DA cell loss over this period, which produced subtle, but significant, behavioral impairments.

Unilateral DA depletion induced by unilateral injection of 6-OHDA along the nigrostriatal pathway elicits different forms of postural imbalance. The widely used motor test for 6-OHDA lesions measures the magnitude of nigrostriatal loss by systemic administration of apomorphine or amphetamine and counting the number of contralateral and ipsilateral rotations, respectively [52]. Such pharmacological approach is reliable and easily reproducible. More recent tests for assessing voluntary asymmetric motor deficits include the cylinder and adjusting step tests [45, 53–57].

Modeling PD Pathophysiology with MPTP Intoxication in Mice

The identification of MPTP, a synthetic heroin that kills DA neurons, led to it becoming among the most widely used toxin to mimic the hallmarks of PD [58]. This is because the toxic metabolite, MPP+, is a potent complex I inhibitor in DA neurons and postmortem PD brains show complex I damage [59]. In nonhuman primates, the effects of MPTP are irreversible and mimic those in humans. In mice, frequent injections and large doses are often required to produce significant DA depletion but the toxin does not always produce large-scale cell death [60, 61].

The MPTP toxin is injected systemically (i.p. or s.c.), destroys 50% of the SN DA neurons and depletes the dorsal, but not the ventral, striatum of DA fibers, thereby mimicking the striatal pathology described for human PD. Investigations have yet to demonstrate extra-nigral pathology, but granular inclusions have been found after the chronic MPTP plus probenecid protocol [18]. MPTP delivery generally follows one of three different protocols: (1) The acute method generally involves four injections in 1 day at 2 h intervals [62, 63]; (2) subacute (also referred to as subchronic) administration is a once-daily injection for 5–8 days [61]; (3) chronic regimens utilize repeated treatments of a month or more and some require implantation of minipumps to deliver MPTP or MPP+, the toxic metabolite [61, 64, 65].

Tyrosine hydroxylase (TH) is particularly sensitive to the MPTP toxin and gene expression for this enzyme is downregulated after toxin administration [66]. Therefore, in order to determine the number of DA neurons that remain after toxin treatment, it is important to count neurons stained for Nissl substance. Using unbiased stereology to count the TH-immunoreactive and Nissl-stained neurons, different labs have shown that a single injection of MPTP (30 mg/kg) induces a 20–30% loss; two injections per day over 2 days lead to a 35% loss and four injections kill approximately half of the DA neurons [67–69]. The chronic MPTP (plus probenecid) regimen produces a rapid but more progressive loss of SN DA neurons, presumably because probenecid retards the clearance of MPTP and its toxic metabolites [58, 61]. With all protocols, striatal DA is dramatically reduced within a week (around 90–95%), with some recovery to 70–80% up to 24 weeks after treatment. Inclusions have been demonstrated with this chronic MPTP/probenecid model, but not other MPTP models [18, 36].

Systemic administration of MPP+ does not damage central DA neurons, because it does not readily cross the blood–brain barrier due to its charge. However, direct infusion of MPP+ for 28 days via an osmotic minipump into the left lateral cerebral ventricle (ICV) produces a dose-dependent, unilateral loss of striatal DA and TH on the side of the infusion. At low MPP+ doses striatal DA is selectively reduced by 37 and 53%, respectively, but higher MPP+ doses produce a greater DA loss (up to 90%). However, the latter also causes significant reductions in serotonin levels. The SN DA cells are reduced by 35%, and over time (2 months later), cell loss is further reduced to 65%. Many surviving DA neurons show ongoing degeneration with silver staining [64, 65]. Inclusions do not seem to be present in remaining DA neurons in this model, but ultrastructural evaluation of the SN showed abnormal mitochondria. One advantage of this ICV model is that it produces a unilateral lesion, thus reducing adverse effects and high mortality rates with bilateral lesions. While the model is technically challenging, it produces a reliable response with little variation, thus making it appealing for testing neuroprotective strategies during the phase of toxic insult and ongoing degeneration, the stage at which PD patients present with the disease.

Behavioral tests in the various MPTP models have been disappointing, and no behavioral assessments have been performed on mice with ICV administration. The Rotarod and open field locomotion are widely employed but are only effective measures of motor deficits if they are administered within a few days of treatment when the mice are still intoxicated by MPTP. More sensitive measures have been able to detect DA loss, such as gait analysis, or the pole, beam walk or grid tests [70].

Environmental Toxins Induce Parkinsonism in Rodents

Chronic pesticide exposure can also lead to the development of PD [71, 72]. Rotenone, which is a naturally occurring pesticide, readily crosses cell membranes and easily penetrates the blood–brain barrier. It has been used to create a chronic rodent model of PD. Rats receive rotenone via osmotic minipumps for up to 5 weeks [73, 74]. Rotenone produces a loss of striatal DA terminals followed by progressive degeneration of SN neurons. Dying DA neurons contain cytoplasmic inclusions, which are immunopositive for alpha-synuclein and ubiquitin [73]. Unfortunately, rotenone either causes selective damage to DA neurons or creates more widespread cell loss [73, 75]. High variability limits the utility of the model [75], but an i.p. route of administration may circumvent these problems. Chronic daily i.p. injections of rotenone reduce striatal DA content and are associated with L-dopa-responsive motor impairments [76]. Behavioral abnormalities are readily detected in the affected rats [73, 75].

Other environmental toxins disrupt mitochondrial respiration and can be used systemically to produce mouse PD models [77]. We know that MPP + and rotenone directly inhibit complex I function, but paraquat (PQ), a herbicide that crosses the blood–brain barrier, disrupts mitochondrial function differently, i.e., via intra-mitochondrial formation of reactive oxygen species. PQ can cause small, but significant, losses of SN DA neurons [78–81]. PQ administration upregulates alpha-synuclein and induces its aggregation [82, 83]. Maneb (manganese ethylenebisdithiocarbamate), a fungicide that inhibits glutamate transport and disrupts DA uptake and release, is generally co-administered with PQ subchronically to enhance toxicity [84–86]. When combined with maneb, PQ can destroy 50% of SN DA neurons in young mice [84], but in older mice (18 months of age), the combined treatment produces a more progressive DA cell loss (approximately 75% at 2 weeks and 88% at 12 weeks) [87]. Motor deficits have not been reliably or readily produced.

Genetic Models of PD

Genetic mutations that are known to cause familial PD can be induced in mice. These mouse models mimic PD, in that many show progressive behavioral

deterioration and increasing pathology with age. Three types of genetic models have recently been developed: (1) those based on the gene deletions that are important for the development or maintenance of DA neurons or their phenotype, such as the Pitx3 or engrail 1 [88–90]; these gene deletions lead to DA cell loss at various times and therefore reproduce the progressive nature of PD; (2) mice with single point mutations or gene multiplication that mimic the familial forms of PD (deficits in alpha-synuclein or other proteins such as parkin, PINK1, DJ1 and LRRK2). Some of these models show a good loss of DA neurons over time, especially those that are created with mutated human alpha-synuclein. (3) Finally, genetic models have been created by virally mediated expression of genes or mutations responsible for familial PD. These models produce a more rapid onset of parkinsonism than the transgenic or knock-out animals.

Summary and Conclusions

There is general agreement that cortico-basal ganglia-thalamocortical abnormality underlies the clinical manifestations of PD [91–93]. In particular, motor deficits have been traditionally associated with a dysregulation of the cortical control of subcortical circuits, which result from the progressive neurodegeneration of the nigrostriatal pathway and chronic depletion of striatal DA. Whether a similar cortico-subcortical mechanism underlies the cognitive deficits in PD remains unclear. The loss of DA in PD, however, is not restricted to subcortical brain regions. DA and its metabolites are also reduced in several cortical areas [94, 95], suggesting that decreases in cortical DA transmission could contribute to the onset of cognitive impairments observed in PD.

Animal models that closely resemble human PD should mimic not only the motor impairments that result from chronic DA depletion of the nigrostriatal pathway but also a progressive lesion of the mesocortical/mesolimbic DA system, which arises in the ventral tegmental area; such lesions may lead to cognitive deficits. Among the different, well-characterized rodent models of parkinsonism, the chronic MPTP/probenecid model stands out as one of the most promising [61, 70], because the pattern of DA cell loss resembles that seen in PD patients [96–98]. With disease progression, the medial portion of the substantia nigra pars compacta and other medial mesencephalic DA cell groups, which are initially spared, become increasingly damaged over time. More importantly, dysfunction of the mesostriatal DA system continues progressively after the chronic MPTP/probenecid regimen is completed [18, 36, 61, 70]. Nevertheless, DA lesions induced by the MPTP/probenecid in young adult ($\sim$2 month old) mice seem to be limited to the substantia nigra, leaving the mesolimbic/mesocortical DA system relatively intact [61]. In older mice (10 month old), however, progressive DA lesion not only affects the nigrostriatal pathway but also compromises the ventral tegmental area [99], as DA

neurons in the mesocortical/mesolimbic system are particularly susceptible in aged animals and humans [100–102]. Future studies are indeed needed to validate this observation and determine whether bilateral mesocortical DA lesion induced by chronic MPTP/probenecid in old mice is associated with cognitive impairments.

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Part II
Physiological Studies of the Cortico-subcortical Dynamics and Parkinson's Disease

Chapter 3
Phasic Dopaminergic Signaling: Implications for Parkinson's Disease

Stefan G. Sandberg and Paul E.M. Phillips

Ever since thediscovery that dopamine is a brain neurotransmitter in the late 1950s [1], its function has been sought after. The first indication of one of dopamine's functions was highlighted by the discovery of the connection between striatal dopamine levels and motor behavior [2]. Given this connection, it is not surprising that Parkinson's disease (PD) has been tied to a dysfunctional dopamine neurotransmission mediated by a preferential neuronal cell loss in the substantia nigra. Indeed, this cell loss is believed to result in a decrease of the ambient, low concentration of extracellular dopamine, called tone, and ultimately the appearance of symptoms in PD [3, 4]. Although dopamine in the dorsolateral striatum (putamen in higher mammals) has been established as being important for normal motor behavior [2, 3, 5–7], dopamine in the ventral portion of striatum, i.e., nucleus accumbens (NAc), has been implicated in reinforcement learning, decision making and some forms of memory [8–12]. Research of dopamine function with techniques capable of subsecond monitoring of neuronal activity has led to the realization that dopamine neuron may communicate with post-synaptic cells through more than changes in dopamine tone. This additional mode of neurotransmission, phasic signaling, is characterized by synchronized burst firing thought to result in a brief increase in extracellular dopamine. In recent studies, phasic signaling in dopamine neurons has been found to occur under several behavioral conditions across different mammalian species [13–20].

This chapter will deal with potential functional implications of phasic dopamine signaling in the context of PD, as empirical observations indicate that phasic dopaminergic signaling is preferentially affected over dopaminergic tone at mild-to-moderate levels of neurodegeneration (0–80%) [21, 22]. Such levels of neurodegeneration would most likely affect dopamine function associated with phasic signaling in the dorsolateral striatum at preclinical and early stages

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of PD (0–80%), but may involve other areas of dopamine innervation as neuronal loss becomes more severe at later stages of PD. The loss of phasic dopaminergic signaling in these other areas, such as dorsomedial striatum (caudate nucleus), NAc and prefrontal cortex (PFC), is likely a major contributor to the cognitive deficits associated with PD [23, 24].

Phasic Dopamine Activation

Phasic dopamine signaling, as described by Grace and Bunney, occurs when dopamine neurons fire action potentials in synchronized bursts of typically three to six action potentials at an interspike interval of $\sim$100 ms (starting at $<$80 ms and increasing during the burst to up to 160 ms) [25]. Grace and Bunney speculated that such phasic activations would lead to short-lasting increases in extracellular dopamine at the terminal regions of midbrain dopamine neurons [25]. Indeed, using in vivo voltammetry, with subsecond temporal resolution, transient changes in extracellular dopamine in response to presentation of a conspecific or cues predicting food or drug availability have been confirmed [18, 19, 26, 27]. Although the existence of phasic burst firing and its subsequent transient rise in extracellular concentration of dopamine at the terminal region is well established, its specific causal role in behavior has still not been fully elucidated.

Phasic firing of dopamine neurons have been found to occur to initiation or the execution of movement, to novel sensory stimuli producing an orientational response or stimuli predicting reward availability. At first, phasic firing was believed to relate to the actual movement itself (direction, amplitude and/or velocity), as phasic activity was found to occur to sensorimotor stimulation (limb manipulations) in anesthetized preparations [28, 29]. However, the view of phasic signaling was expanded following seminal studies conducted by Schultz and colleagues demonstrating enhanced phasic activation when movement was paired with reward [13, 30]. They found that dopaminergic neurons respond to unexpected delivery of either rewards or reward-predicting stimuli [13, 14, 30]. This pattern of activation is such that there are phasic increases in the firing rate when rewards are better than expected, or reward-predicting stimuli are unexpectedly presented; and there are phasic decreases in firing rate (pauses in firing) when rewards are worse than expected or when conditioned inhibitors (stimuli that predict the absence of a reward that would otherwise be available) are presented. However, phasic dopaminergic activity has been observed for other arousing stimuli, leading to the suggestion that its role may extend beyond reward-related behavior [15, 31].

Purported Functions of Phasic Dopaminergic Signaling

Many different functions have been ascribed to phasic dopaminergic signaling since its discovery. Redgrave et al. have argued that the latency to onset of phasic bursting of dopaminergic neurons is too short to subserve any higher

cognitive function, such as specific evaluation of pending rewards, since phasic bursting occurs before orientation to the stimulus [32]. In this hypothesis, phasic dopamine signaling is viewed as shifting the behavior of a certain task to another [32], and may be a remnant from an evolutionarily older thalamostriatal circuit [33]. This short-latency phasic dopamine release might have served as a modulator of associations between less defined sensory input and appropriate motor output. An example of such a motor output would be an orienting response for stimulus identification, like saccades.

However, in contrast to this, empirical data have shown that short-latency, phasic signaling of dopaminergic neurons scales to the magnitude [34] and probability [35] of the predicted future reward, demonstrating unequivocally that a component of economic value can be encompassed in the dopaminergic response within just ~100 ms of the stimulus onset. These data suggest that the dopamine response represents a cached value of the unexpected stimulus (see below). More parsimonious with these data, Redgrave and Gurney have recently proposed an additional function of the short-latency phasic dopaminergic signaling in agency detection [36]. In this model phasic dopamine is thought of as a reinforcer for motor behavior preceding a reinforcing sensory event. In this way an agent/animal can identify what action (through the dorsal striatum) or what context (through the ventral striatum) a reinforcer was delivered [36, 37].

These electrophysiological and other experimental data have stimulated several intriguing ideas regarding dopamine function. For example, the incentive salience hypothesis posits that dopamine signals encode an incentive value for rewards and/or cues associated with rewards [38]. Here the level of phasic dopamine could correspond to the degree of wanting of a certain reward. Along similar lines, the lab of Salamone has collected interesting data indicating a role for dopamine as a behavioral activator by acting as a substrate for motivation. For instance, with dopamine depletion, effort-based decision making appears to be biased away from instrumental responding requiring high effort, i.e., energy expenditure [39].

In keeping with this, one of the functions ascribed to phasic dopamine signaling is action selection [40]. The functional anatomy of the basal ganglia along with dopamine release in the striatum is believed to aid in promoting a motor behavior of desired intent while inhibiting competing motor behavior [41–44]. The desired motor program (action plan) is presumably represented by the highest intensity of cortical input to the striatum and is further distinguished from competing motor programs by dopamine enhancing the signal (desired motor program) to noise (competing motor programs) ratio at the level of the medium spiny neurons. Here, phasic dopamine release could act as a temporally specific indicator as to which signal to enhance over noise. Several electrophysiological studies have identified such a function for dopamine in the NAc as well as the PFC [45].

When thinking about how the phasic dopamine signals might be involved in appetitive behaviors (motivation and action selection), one thing to bear in

mind is that reward-related phasic firing of dopamine neurons is not simply a report of the current or predicted reward but occurs only during a mismatch of the previous reward prediction and new sensory signals (either reward predictors or rewards themselves). This is described as a prediction error [46] and formalized as the difference between the reward received and the reward expected. Interestingly, in the field of animal learning, Rescorla and Wagner described a process by which animals could update the associative strength of environmental stimuli using such prediction errors [47]. This model was a major advance over existing theories since it could account for learning anomalies such as the blocking phenomenon [48]. The use of prediction errors to drive learning is also a feature of models in the field of machine learning. Notably, the temporal difference reinforcement learning (TDRL) model is a time-derivative algorithm that uses a prediction error to update the associative strength between stimuli [49]. Indeed, the match is remarkable between the prediction error variable from this model and phasic dopamine neuronal activity measured under a variety of learning conditions [14, 16, 35, 46, 50, 51].

Within the TDRL model, the expectation of reward is tracked over time during the presentation of arbitrary or rewarding stimuli. The expectation signal is updated upon stimulus presentation based on the reward-predictive history of the stimulus. The expectation signal accounts for partial predictors of rewards (those that are probabilistic predictors) by scaling the predicted reward magnitude (if a reward is delivered) by the predicted probability of reward, thus capturing the average reward expectation (known as expected value in the field of economics). The purpose of the TDRL model is to update the predictive weighting or value of stimuli at times when the expectation was not met. To do this it uses a prediction error signal which equates to the time derivative of reward expectancy. Therefore "errors" are reported when better or worse than expected rewards are delivered, or when predictors of reward are unexpectedly presented. It has been demonstrated under several conditions that phasic dopamine activity agrees with the change-in-expectation "teaching" signal generated by this model [14, 16, 35, 46, 50, 51]. In its simplest form the stimulus value is updated by phasic dopamine signals – it is increased when reward presentation was better than expected (phasic increases in dopamine neuron firing), and decreases when the reward was worse than expected (phasic decreases in dopamine neuron firing).

However, it is considered that multiple systems, encompassing different computational complexities, are orchestrated to guide reinforcement learning. As such, any one reinforcement learning model does not universally capture all aspects of reward-related learning. The TDRL model is thought of as a cached-value-based system [52]. That is, predictive stimuli acquire "value" based on their history of pairing with reward. Importantly, this model is not a declarative system, so there is not an iterative process of recognizing the stimulus and then consciously deriving that certain rewards follow the stimulus. Rather, it is more like the stimulus has value even though the organism does not necessarily know why. This feature is consistent with the idea of incentive attribution to

reward-predicting cues [38, 53]. This process allows a fast reward-predicting system with low computational demand. On the other hand, there are alternative reward-predictive models that are more computationally complex, such as those described by tree searching. This is a more deliberative process where all the possible outcomes following the stimulus are computed when the stimulus is presented. Prefrontal cortical regions [52] and hippocampus [54] have been implicated as neural substrates for tree-search-based models.

In addition to classical conditioning, TDRL algorithms have been applied to instrumental and procedural learning, where actions optimizing reward is of primary concern. Under these learning paradigms the TDRL model is framed within the concept of an actor-critic architecture [55–57]. Here, the actor is thought to be the cortico-basal ganglia loop, where information regarding actions associated with potential reward acquisition is transmitted. The critic is the midbrain dopaminergic neurons, signaling prediction errors for actions (as opposed to stimuli) resulting in reward. In this way the actor-critic model is thought to be involved in sensorimotor learning and indeed appears to support learning of action sequences leading to delayed reward [55, 58]. As mentioned above procedural learning has been captured by the actor-critic model, which may provide a theoretical framework for the symptomology of PD. Deficits in sequential movement have been observed as well as cognitive deficits in early and later stages of PD [23, 24, 59–61]. In order to assess the potential impact of decreased phasic signaling as a result of dopaminergic neurodegeneration, it may be of interest to first highlight normal functions associated with areas of dopaminergic innervation and its neurotransmission.

Nucleus Accumbens and Associative Learning

Many functions have been ascribed to the striatum, e.g., associative and procedural learning and action selection. According to learning theory the early stages of learning is characterized by goal-directed behavior and as such is sensitive to the outcome. Moreover, evidence indicates that the striatum is subdivided into functional subdomains [24, 62]. Lesion studies and pharmacological inactivation of dopaminergic neurons terminating in the ventral striatum, or more precisely NAc, have demonstrated that NAc dopamine is important for action–outcome associations in goal-directed behavior, a characteristic of instrumental conditioning [62–64]. Action–outcome associations are important as it represents the formation of a connection between an action or behavior and its resulting outcome or consequence. This type of association is flexible and outcome dependent, i.e., it becomes interrupted by no and/or devalued rewards being delivered. Moreover, this association allows you to adapt your behavior in a changing environment. In recent development of the reinforcement learning field, TD learning has become the more dominant approach when explaining learning. Again this trend is presumably due to the finding that

dopaminergic neurons fire in a manner consistent with prediction errors, a teaching signal adopted from the machine learning field [55]. This signal is thought to update reward expectancy in the biological model of learning and represents a way for an organism to adapt to changing reward contingencies in the environment.

At a cellular level dopamine is a modulatory neurotransmitter and has been shown to mediate synaptic plasticity both morphologically [65] and electrophysiologically [66–69], the phasic activation of dopaminergic neurons is thought to be a signal that facilitates learning by enhancing synaptic weights at the glutamatergic synapses of striatal medium spiny neurons. In fact, stimulating electrode placement in the substantia nigra, presumably causing phasic dopamine release in the striatum, has been shown to support intracranial self-stimulation and to cause long-term potentiation in a D1 receptor-dependent manner [68]. Such long-term synaptic changes may underlie a cellular mechanism for the memory of learned associations.

Dorsolateral Striatum and Stimulus–Response Associations

Whereas the ventral striatum is associated with stimulus–stimulus associations, the dorsolateral striatum has been implicated in stimulus–response associations. This type of association is characterized by inflexibility and outcome independence. A behavior driven by stimulus–response association is no longer adapting to changes in reward contingencies in the environment and can appear to happen without obvious external reason. However, this association happens at a late and overtrained stage when reward contingency has been repetitively sampled for an extended time, and as such is therefore well known. Once an action leading to a well-predicted reward delivery is identified it would be maladaptive to stop this action if reward would not be delivered occasionally. Thus, stimulus–response associations may allow for perseverance on a successful behavioral strategy and renders an organism insensitive to occasional reward omissions. Stimulus–response association may also be important for procedural memory or action sequence memory [62, 64, 70–72]; again the actor-critic architecture is especially suitable for this type of learning and memory formation [55]. One way in which phasic signaling is thought to mediate procedural learning is through chaining of individual actions within a sequence. Initially, there would be a phasic signal to reward delivery and upon repeating the reward yielding action sequence, the phasic signal would first transfer to the most proximate action, in relation to reward delivery. Upon further repetition the phasic signaling of dopaminergic neurons would transfer to more and more distal actions within the sequence until it has reached the first action (temporally the most distal in relation to reward delivery). At this stage, the signal at the first action event now signals for the upcoming delayed reward and initiates the chain of actions to achieve reward. Whereas actor-critic models capture

procedural reinforcement learning it may not be as clear as to how procedural learning would occur when intrinsic stimuli or goals are driving the behavior. Indeed as we will see, it may be this aspect of procedural learning and memory that is preferentially affected in PD. However, another feature of PD symptomology is increased perseverative behavior [59], This symptom is a general descriptor of a maladaptive behavior that can have several sources. One of which is a denervated striatum, but also a denervated prefrontal cortex.

Prefrontal Cortex and Working Memory

Areas of the PFC are important neurobiological substrates of executive function including working memory, sensory-motor gating and decision making, in higher mammals [73, 74]. Working memory is important for the ability to hold and manipulate information when involved in problem solving and decision making [75]. Dopamine innervation to these regions is critical for normal function, both at the cellular and behavioral levels [76–81]. In particular, D1-receptor activation has been shown to stabilize neuronal ensembles with high activity in the PFC. This persistent and stabilized state of neuronal activity has been suggested to represent the maintenance of information held as working memory [82]. Conversely D2-receptors appear to have the opposite effect in that they destabilize neuronal ensembles, thus rendering them more susceptible to interfering neuronal input. This state is thought to be involved in the updating of new information to be held in working memory. Indeed computational models have captured this aspect of the prefrontal cortex [83]. Here, strong phasic dopaminergic input is thought to first activate the low-affinity D2-receptors gating for new information input and subsequently activate high-affinity D1-receptors acting to maintain the newly acquired information in working memory [82]. An additional function ascribed to the prefrontal cortex is attention. This cognitive function is primarily investigated by observing subjects ability for attentional set shifting, which is the ability to respond to changes in stimuli dimensions that are predictive of reward. Furthermore, imaging and lesion studies have implicated the prefrontal cortex with its connections to the putamen (dorsolateral striatum in rats) in attentional set shifting [23]. This is demonstrated in the study of Cools et al. where striatal dopamine is necessary in order for task set shifting to remain intact; as evidenced by set-shift impairments upon the cessation of dopamine replacement therapy in volunteering PD patients [23].

Although working memory may have its focus in the prefrontal cortex, the complete function likely derives from an interaction between associated cortical and basal ganglia structures. As we shall see in the following section dopamine denervation in the putamen of Parkinsonian patients or experimentally induced lesions to that area results in cognitive symptoms akin to frontal lobe dysfunction [84]. An interesting theoretical model (prefrontal cortex, basal ganglia working memory, PBWM model), capturing the potential importance of such

an interaction, is described by O'Reilly and Frank [74]. Frank and colleagues demonstrate the importance of an intact basal ganglia and prefrontal cortex for normal working memory processing. Furthermore, the suggested interaction between the basal ganglia and the prefrontal cortex allows for adaptive updating of the working memory by the basal ganglia, through reinforcement learning [85]. The phasic signaling of dopaminergic neurons associated with reward delivery is proposed to result in gating of sensory information into working memory. In this way, working memory will obtain and maintain sensory input that leads to positive outcomes [85].

Dopaminergic Signaling and PD

The hallmark of PD pathophysiology is the loss of dopaminergic neurons of the midbrain. This denervation has been associated with a decrease in the ambient steady-state concentration of extracellular dopamine known as the dopaminergic tone. Indeed animal studies utilizing microdialysis have correlated the loss of tone with the appearance of the classic symptoms of PD, i.e., resting tremor, rigidity and bradykinesia or slowness of movement [3, 4]. However, PD provides a remarkable example of neurological homeostasis as the dopaminergic tone does not drop until the striatal dopamine tissue content has decreased with 80%. Many compensatory models have been proposed to explain the absence of symptoms despite neuronal loss, and perhaps the most dominant one has been proposed by the lab of Zigmond [4]. Here they have proposed that surviving dopaminergic neurons compensate by increasing the synthesis and release of dopamine and decrease in uptake. These changes in dopamine neurotransmission are thought to result in an increased level of dopamine in innervated regions which subsequently diffuses to post-synaptic neurons in denervated regions, and thus maintain normal dopamine neurotransmission during PD. However, recent studies with high-temporal-resolution fast-scan cyclic voltammetry, which can dissociate release and uptake, demonstrated that no compensatory changes in these processes are present following striatal denervation in a rodent model of PD [21, 22, 86]. These results present an apparent conundrum in that dopamine tone is maintained despite no compensatory increase in release or decrease in uptake. However, this lack of active neuroadaptation can be accounted for by a new compensation model of preclinical parkinsonism called passive stabilization [21, 22, 87].

The salient observations that inspired the passive stabilization model are that (1) dopamine tone is a steady-state concentration maintained by a balance between release and uptake and (2) dopamine release and uptake in the striatum are decreased co-linearly following denervation in proportion to denervation level. In retrospect, the latter observation is intuitive since high-affinity dopamine uptake is mediated by the dopamine transporter which is exclusively expressed in dopamine neurons. Thus, the loss of dopamine terminals renders the depletion of both dopamine release sites and dopamine uptake sites in

proportion. The observation of steady-state concentration was made during empirical measurements of dopamine [86, 88, 89], but is also predicted by mathematical models that simulate extracellular dopamine concentration based on release and uptake kinetics [90]. The passive stabilization model describes the interaction of co-linear loss of release and uptake during denervation with the level of the steady-state dopamine concentration. From measurements across a range of denervation states, Garris and colleagues determined that the steady-state concentration is preserved until more than 80% of striatal dopamine tissue content is lost, without the need for any active compensation whatsoever [21]. Because of the co-linear loss of release and uptake sites, the ratio between these processes is maintained resulting in an unaltered steady-state tone. It is only when neuronal loss is so extreme (>80%) that passive stabilization is no longer maintained and there is a diminution of tone.

Although the loss of tone is commonly accepted as the pathophysiological consequence of dopamine denervation, less is known about the effect of dopaminergic cell loss on phasic dopaminergic signaling. According to voltammetry studies it appears that phasic signaling is not maintained; in fact it decreases in proportion to denervation [21, 86]. This was demonstrated by a decrease in the amplitude of dopamine release evoked by electrical stimulation of the medial forebrain bundle [21, 22]. It may appear contradictory that dopamine tone and not phasic signaling is compensated. This was explained by Bergstrom and Garris by considering that the amplitude of dopamine tone is dependent on both release and uptake, whereas the amplitude of phasic signals is primarily thought to be dependent on release alone [21]. Thus, since release decreases in proportion to denervation so will the amplitude of phasic signals. It would be surprising if this apparent uncompensated aspect of dopamine neurotransmission did not have behavioral consequences in the preclinical phase of PD (0–80% denervation) as long as it remains uncompensated at the cell body level both in the midbrain and/or the target neurons of the striatum. However, recent evidence indicates that some level of compensation may exist in response to decreased phasic signaling in the preclinical lesion range, as measured by "time-share" voltammetry and electrophysiology [91] allowing for detection of dopamine release and resulting single unit activity of striatal units [92]. This compensation at the striatal level appears to be primarily mediated by the D2-receptor. However, clear evidence for a decrease in the amplitude of phasic signaling when dopaminergic tone remains intact does exist and may add an intriguing piece of the puzzle to the overall pathophysiology and symptomology of PD.

Parkinson's Disease, Motor and Cognitive Impairments, and Phasic Dopaminergic Signaling

Parkinson's disease is perhaps best known for affecting motoric behavior. Considering that the primary site of dopaminergic denervation is the putamen in PD, it is not surprising to find the most obvious motor deficits in tasks

demanding sensorimotor integration [93]. However, a wide array of cognitive symptoms has also been associated with PD ranging from attentional set shifting to sequence learning deficits [23, 59, 84]. It is noteworthy that cognitive symptoms often appear in the early stages of PD, when tonic levels of dopamine may still be relatively intact, which in turn would indicate a possible role for phasic signaling as the culprit for these symptoms. Indeed, that is what the passive stabilization model of neurochemical compensation during PD predicts, i.e., that phasic dopaminergic signaling is preferentially affected by mild to moderate dopamine neurodegeneration [21, 22, 87]. An alternative explanation would be that cognitive symptoms associated with PD are not mediated by dopaminergic cell loss, but instead due to degeneration of other nuclei [94]. However, this seems unlikely to be the cause of cognitive symptoms as dopamine replacement therapies ameliorate these symptoms [24].

The neurodegeneration of PD occurs in a topographic manner over time. The area affected the most and earliest is the putamen and the dorsal tier of the caudate nucleus (homologous to the dorsolateral and dorsomedial caudate–putamen in rats, respectively). As the disease progresses the ventral striatum and prefrontal cortex are both subject to dopaminergic denervation [95–98]. Thus, the topographical and temporal progression of the neurodegeneration result in an appearance of symptoms associated with each respective area in time.

Neurodegeneration of the putamen results in the loss of sensorimotor integration [93, 99, 100]. For example, visual and proprioceptive-motor integration and visuospatial working memory appear to be affected in PD [93, 99, 101]. What role phasic signaling may play in such deficits is not exactly known; however, here we offer an exploratory model of sensorimotor integrative deficits. In order to answer the question of how voluntary movement breaks down in PD, one should perhaps first ask the question how voluntary movement is formed.

Here we present a new view of voluntary movement in the context of proprioceptive space. The concept of proprioceptive space is a cognitive psychological premotor theory of consciousness. However, for the purpose of this chapter we will use it to explain voluntary movement deficits in PD. Proprioceptive space (PS) is a three-dimensional coordinate system around a person that is within limb reach. It is the visual and proprioceptive inputs associated with past motor activity to a particular coordinate that form PS [102]. In order to associate a certain motor program to a particular point in the PS it is necessary to have a teaching signal that indicates correct outcomes. Phasic activity in dopamine neurons may operate as such a teaching signal. In the initial stages of associating a motor program with a desired point in PS, the phasic dopamine signal would indicate whether the point was reached or not, as judged by visual and proprioceptive feedback. In a way, this stage of learning could be said to be a stimulus–stimulus association. As a motor program is repeated to a particular point over time, PS becomes predominantly associated with the movement itself or action, and thus may be said to exist as a stimulus–response association. Unlike volitional movements, the results of stimulus–response associations are thought of as subconscious actions, occurring without need for specific

attention. In other words proprioceptive space can be viewed as a motor program memory representation of immediate reachable space. Indeed, stimulus–response associations have been found to be dependent on an intact dorsolateral striatum of rats [62, 64, 72, 103], which is the area primarily degenerated in PD. Within the context of this model, an absence of phasic dopaminergic signaling could lead to an inability to form new and maintain existing stimulus–response representation of PS. In this case movements within the PS would revert back to being driven by stimulus–stimulus associations between visual and proprioceptive input and motor output. Movements of this type would be outcome dependent, and for optimization purposes, iterative in nature. We submit that this slows voluntary movement down as one has moved from a subconscious movement in space to being dependent on constant visual and proprioceptive feedback. More specifically, the loss of cache-based learning systems would require that actions become more deliberative.

An additional deficit PD patients express is the inability to form new sequential memories or procedural memories [104, 105]. This may be as a result of decreased amplitudes of phasic signals, thought to be necessary for linking or chaining several behavioral sequences to a delayed reward. As an additional consequence of decreased ability to form new procedural memories, one might hypothesize that PD patients would display inflexibility in sequential behaviors, as they would have a hard time adapting to new contingencies in their environment. This inflexibility can perhaps be viewed as a form of perseveration, another symptom observed in PD [59]. Moreover, not only is there inflexible behavior associated with deficits in sequence learning, but also in such cognitive tasks as attentional task set shifting. PD patients have demonstrated an inability to shift their attention from one stimulus dimension to another when prompted by a cue [23]. Again, the underlying theme appears to be an inability to respond to a new contingency and similarly this would appear as a perseveration. The cortical area(s) important for such task set shifts have been shown to connect to the caudate nucleus in non-human primates [84], an area affected in PD.

Perseveration could perhaps also be explained by deficits in working memory. According to the working memory model of Durstewitz and Seamans, activation of low-affinity D2-receptors in the PFC is necessary for updating working memory, while high-affinity D1-receptor activation is important for maintenance of working memory. In this model D2-receptor activation is thought to be mediated by dopaminergic phasic signaling. A decrease in the concentration of phasic signals would lead to less D2-receptor activation and hence decreased ability for updating working memory. Meanwhile D1-receptor activation, dependent on dopaminergic tone, would remain intact. In such a scenario a bias for maintenance of existing information in working memory would be prevalent, again potentially appearing as perseverative behavior. However, degeneration of mesocortical dopaminergic neurons only occurs at the later stages of PD [98].

Conversely, another important function of the putamen or dorsolateral caudate–putamen is thought to be the formation of stimulus–response associations, which can be viewed as an association with a habit-like inflexibility. Such inflexibility could again be said to possess perseverative qualities. Here decreased phasic signaling would prevent stimulus–response associations to be formed in favor of more outcome-sensitive associations with their locus in the ventral striatum. Indeed, this observation has been made in a 6-hydroxydopamine model of PD in rats [62]. In this case, degeneration of the putamen would prevent perseverative behavior. Another group of dopaminergic neurons affected by degeneration in severe PD is the mesolimbic neurons. As mentioned above phasic signaling in the ventral and medial aspect (caudate nucleus in higher mammals) of the striatum is thought to be important for the formation of stimulus–stimulus or action–outcome associations, respectively [62, 106]. Consequently, decreased phasic signaling in these areas would impair the formation of such associations. Consistent with this, mice with a conditional knockout of the NR1 subunit on dopaminergic neurons have been found to lack phasic signaling and display impaired learning (stimulus–stimulus associations are retarded) (Zweifel et al., in submission). Furthermore, denervation of the striatum has been shown to decrease learning from positive feedback of a probabilistic selection task in PD patients [24]. This task is characterized by new and flexible learning and as such would be considered an action (the selection)–outcome (reward) association. Furthermore, positive feedback is thought of as being mediated by phasic dopaminergic signaling and in this task would primarily be dependent on the medial (caudate nucleus) and ventral (nucleus accumbens) striatum. Again, the impaired learning is thought to be caused by decreased phasic signaling, thus resulting in "weaker" feedback as to which is the best option. Another effect of decreased amplitude of phasic signals is a potential loss in the dynamic range, hence making the difference between a good versus a bad choice, neurochemically, small to insignificant. A deficit of this nature would make decision making more difficult and perhaps result in indecision.

Conclusion

PD disease was originally described by James Parkinson [107] as a motoric disease with "senses and intellect being uninjured". Since then a plethora of cognitive symptoms associated with PD has been discovered [98, 101, 108]. The motor symptoms of PD have been associated with decreased dopaminergic tone. However, the symptomatic picture of both motoric and cognitive symptoms has been difficult to explain with deficits in tone alone, and has not incorporated phasic dopaminergic signaling until recently. In this chapter we have highlighted a new neurochemical compensatory model, developed by Garris and co-workers. This model captures the main compensated feature of

PD, maintained dopaminergic tone, but also a new and intriguing deficit in phasic dopaminergic signaling. Furthermore, we have used this deficit in phasic signaling along with purported normal function as a framework for making predictions on how the three main areas utilizing phasic dopaminergic signaling would be affected from a behavioral perspective. This chapter is by no means all inclusive, but has hopefully introduced the reader to a new revised idea regarding the pathophysiology of PD (deficit in phasic dopaminergic signaling) and its symptomology (both motor and cognitive).

It may seem odd that no obvious symptoms appear to exist in the preclinical phase of PD (0–80% striatal denervation) despite a loss of phasic signaling. One has to keep in mind that the brain, in general, can display powerful compensatory mechanisms rendering symptom detection difficult. Moreover, some symptoms associated with PD appear to be memory loss, which in turn could be dependent on phasic signaling for their maintenance. Losing phasic signals gradually, as proposed by Garris and co-workers, would result in a gradual loss of memory gradual as well and allow time for potent compensatory mechanisms. Such compensatory mechanisms will not only present challenges for early detection of PD but can also lend a hand in the discovery of brain function and sites for pharmacological intervention.

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Chapter 4
Striatal Dendritic Adaptations in Parkinson's Disease Models

Michelle Day and D. James Surmeier

Introduction

Parkinson's disease (PD) is a widespread and disabling neurodegenerative disorder that results from the loss of the dopaminergic innervation of the striatum [1]. Dopamine (DA) depletion triggers an array of biochemical and structural adaptations, some of which are compensatory but others of which appear maladaptive.

The principal neuronal cell type in the striatum and the principal target of the dopaminergic innervation lost in PD are the medium spiny neuron (MSN). MSNs can be divided into two roughly equal groups based on axonal projections, peptide expression and their expression of dopamine (DA) receptors [1, 2]. MSNs that preferentially project axons to the substantia nigra express D_1 DA receptors, whereas those that preferentially project to the external segment of the globus pallidus express D_2 DA receptors [3, 4]. Early work suggested that the activity of MSNs increased in animal models of PD. For example, Schultz et al. noted that the spontaneous activity of presumptive MSNs was elevated following 6-OHDA lesioning [5]. Subsequent studies confirmed this observation and showed that recovery of motor function was correlated with diminished spontaneous activity [6]. Intracellular recordings from unidentified MSNs following unilateral 6-OHDA lesioning of the dopaminergic innervation of the striatum have reported more depolarized membrane potentials and increased synaptic noise [7, 8], in agreement with the earlier studies.

However, indirect measures of neuronal activity (e.g., IEG, peptide expression) suggested that the effects of DA depletion were cell-type specific, resulting in diminished activity in striatonigral and increased activity in striatopallidal MSNs [1, 9, 10]. Elevated activity levels of the internal segment of the globus pallidus and subthalamic nucleus in PD patients and animal models of PD are

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consistent with this view [11–13], although there are a number of observations that are difficult to reconcile with this simple picture [14–17]. Recent work in vivo has provided direct support for this cell-type-specific model [18]. Using extracellular recording and antidromic identification in rats, Mallet et al. [18] showed that the spontaneous activity of presumed striatonigral neurons was lower 3–4 weeks after a 6-OHDA lesion, whereas it was increased in nominal striatopallidal MSNs. This change in spontaneous activity was attributed in part to shifts in the responsiveness to cortical stimulation, as well as a depression in the activity of corticostriatal neurons preferentially forming synapses on striatonigral MSNs.

A major obstacle to resolving the adaptations in MSNs has been the inability to reliably distinguish them in either in vivo or in vitro recording paradigms. This situation has now changed, at least for in vitro work. Each of these MSN populations now can be reliably sampled in bacterial artificial chromosome (BAC) transgenic mice in which green fluorescent protein (GFP) is expressed under control of D_1 receptor or D_2 receptor promoter regions [19, 20].

This chapter will focus on studies conducted in the last few years using this new tool. *These studies have confirmed that the adaptations in MSNs are cell-type specific and intimately connected to alterations in dendritic excitability* [20, 21].

MSN Dendrites are Excitable

The dendrites of MSNs are the primary target of the striatal dopaminergic innervation [22]. Yet, relatively little is known about how the physiological properties of MSN dendrites and how they are modulated by DA. A major obstacle to gaining a better understanding of these regions is their small size and non-planar organization. Optical approaches, particularly 2-photon laser-scanning microscopy (2PLSM), offer a powerful strategy for probing dendritic function, particularly when used in conjunction with somatic patch clamp recordings. This approach has recently been used to study proximal MSN dendrites in brain slices [23, 24]. We have recently extended these observations to more distal dendritic regions of MSNs. These studies support four basic conclusions. First, backwardly propagating action potentials (bAPs) are actively propagated in the proximal dendritic trees of MSNs, but appear to be passively propagated into more distal dendritic regions; this propagation is more robust in D_2 MSNs than in D_1 MSNs. Second, the propagation of potential changes produced by bAPs is actively shaped by dendritic K^+ channels, most likely Kv4 channels. Third, the dendritic Ca^{2+} signal associated with a single bAP is modulated both by focal application of both DA and ACh receptor agonists in D_2 MSNs, but this bAP-associated signal is not reliably modulated in D_1 MSNs. Lastly, DA depletion increases the dendritic Ca^{2+} signal associated with bAPs in D_2 MSNs, adding an important new insight into the mechanisms underlying striatal adaptations in PD [25].

Individual action potentials generated at the soma produced reliable Ca^{2+} transients in proximal (~30–50 μm from the soma) dendritic shafts and spines of both D_2 and D_1 MSNs. These bAP-evoked Ca^{2+} transients were also reliably

detected in more distal (~100 μm from the soma) dendrites and spines of the D_2 MSNs. The relative amplitude of this fluorescence signal fell with distance beyond about 50 μm from the soma, presumably because the amplitude of the bAP-associated potential change also fell with eccentricity from the soma [26, 27]. The rate at which this signal fell with distance was significantly greater in D_1 MSNs than in D_2 MSNs (Fig. 4.1). The decrementing fluorescence signal

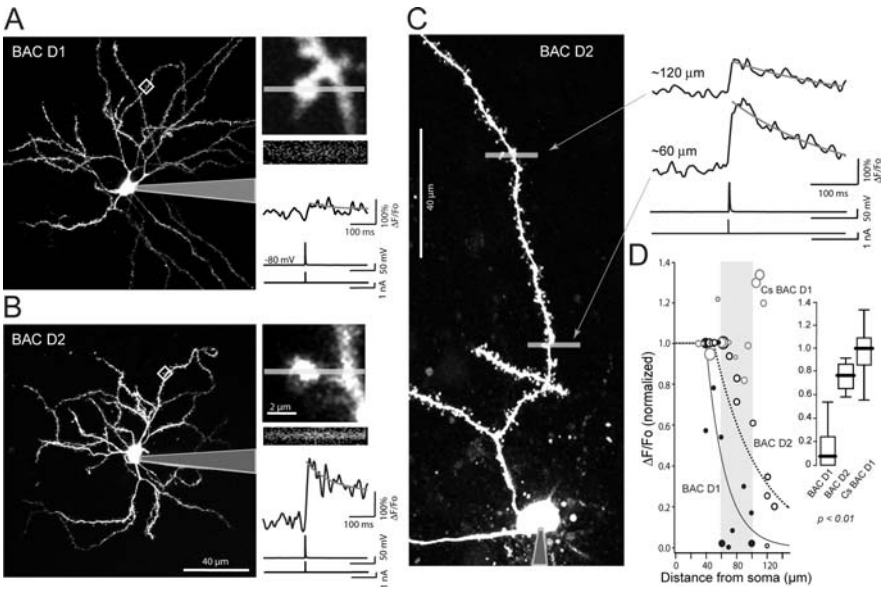

Fig. 4.1 BAP-evoked Ca^{2+} transients are readily detected in the distal dendrites and spines of the D_2 population of MSNs. (**A, B**) 2PLSM images of MSNs in 275-μm-thick corticostriatal slices from a (**A**) BAC D_1 and (**B**) BAC D_2 mouse. Neurons were visualized with Alexa Fluor 568 (50 μM) by filling through the patch pipette (patch pipettes are *grayed out* for presentation). Maximum projection images of the somas and dendritic fields (left panels **A** and **B**) and high magnification projections of dendrite segments from the regions outlined by the *yellow boxes* are shown (*top right panels* **A** and **B**). BAP-evoked Ca^{2+} transients were detected by line scanning through the spine in the region indicated by the *gray line*. Fluorescence traces were generated from the pseudocolor image (lower panels **A** and **B**) by calculating $\Delta F/F_o$ (*top black trace*). The fluorescence image, $\Delta F/F_o$ trace, action potential (*middle trace*) and current pulse (*bottom trace*) are shown in temporal registration. (**C**) Maximum projection image of a soma and dendritic branch from a D_2 MSN. Line scans were acquired at two eccentricities, 120 and 60 μm, as indicated by the *gray arrows*. (**D**) Graph of the change in amplitude with distance from the soma calculated by normalizing scans taken at distal points to the most proximal scan point in each MSN. The magnitude of the Ca^{2+} transients decrements more in the D_1 MSNs (D_1 MSNs, *filled black circles*; D_2 MSNs, *open black circles*). This decrementation is not seen in MSNs loaded with Cs^+-based internals (*open gray circles*). The points were scaled to represent the number of cells scanned at each point (smallest points, 1 cell; largest points, 4 cells). The data, fit from the median distance of the most proximal point, show that the magnitude of the Ca^{2+} transients decrements more in the D_1 MSNs ($n = 11$, *black line*) vs. the D_2 MSNs ($n = 6$, *dashed line*) [Kruskal–Wallis ANOVA, $p < 0.01$]

was not attributable to a reduced density of Ca^{2+} channels at these sites, as increasing the somatic depolarization or improving distal voltage control by filling cells with Cs^{+} allowed large Ca^{2+} signals to be generated right to the visible tip of the dendrite. Rather, the decrementing Ca^{2+} signal was likely due to decrementing propagation of the bAP-evoked potential into distal dendrites lacking Na^{+} channels. This inference is drawn from the observation that application of the Na^{+} channel toxin TTX to the proximal dendrites virtually eliminated more distal bAP-evoked elevations in Ca^{2+}-dependent fluorescence (indicating active propagation of the bAP through the proximal dendrites), whereas application of TTX to distal tertiary dendritic locations (>60 μm from the soma) had virtually no effect on bAP-evoked fluorescence changes. Computer simulations using a model that captured key features of the MSN geometry and channel expression confirmed that in tertiary dendrites lacking Na^{+} channels bAPs declined in amplitude as they traveled away from the soma. But these simulations also suggested that, at least within the initial portion of the tertiary dendrites, the amplitude of the bAP was still sufficient to activate relatively low threshold Cav1.3 or Cav3 Ca^{2+} channels.

Why there might be a greater attenuation of bAP propagation into the dendrites of D_1 MSNs is not obvious. Studies in other neurons have shown that dendritic geometry is an important factor governing bAP propagation [28, 29]. However, geometric differences in the dendritic trees of MSNs have not been described. Furthermore, recent work by our group indicates that the branching structure of dendrites is not different in D_1 and D_2 MSNs (Getler, 2008 in press). Another factor governing bAP propagation is the ion channel investment of the dendrites. Voltage-dependent Na^{+} channels support bAPs, helping to maintain the amplitude of bAPs as they invade dendrites [26]. Voltage-dependent K^{+} channels, on the other hand, oppose bAP propagation [30]. Kir2 K^{+} channels are robustly expressed in MSN dendrites [21, 31]; however, inwardly rectifying Kir2 channels rapidly block at potentials above the K^{+} equilibrium potential, making them poor regulators of bAP propagation. In other neurons, depolarization-activated Kv4 channels have been shown to be potent bAP regulators [30]. Our work revealed that the dendrites of MSNs are also invested with Kv4 channels, in agreement with previous scRT-PCR studies showing Kv4.1-3 mRNA expression in MSNs [32]. Moreover, block of Kv4, but not Kv1, channels enhanced bAP-evoked dendritic Ca^{2+} signals. Simulations of bAP propagation suggested that reducing Kv4 density by half could readily account for the change in dendritic bAP-evoked Ca^{2+} signals. However, because the pharmacological tools available are not selective, alternative approaches will be necessary to unequivocally determine the role of Kv4 channels in MSN dendrites [33].

DA Suppresses While ACh Enhances Dendritic Excitability in the D_2 MSNs

Asymmetries in the neuromodulatory effects of DA on striatopallidal and striatonigral MSNs have long been inferred from their differential expression of D_1 and D_2 receptors. D_1 receptor stimulation generally enhances the

response to excitatory inputs, whereas D_2 receptor stimulation attenuates responses to excitatory stimulation [34–36]. Our results point to another example showing that local application of DA diminishes bAP-evoked Ca^{2+} signals in the dendrites of striatopallidal D_2 MSNs, but has no detectable effect on the same response in striatonigral D_1 MSNs (Fig. 4.2). The D_2-receptor-mediated response in striatopallidal MSNs is consistent with their negative coupling to both Cav1 and Cav2 Ca^{2+} channels likely to underlie the bAP-evoked response [37–39].

The absence of a dendritic response to DA application in D_1 MSNs is somewhat surprising. D_1 receptor stimulation promotes the slow inactivation of Na^+ channels in MSNs [40, 41]; however, because slow inactivation occurs only at depolarized potentials, our experimental paradigm was not suited to bringing out this modulation. D_1 receptor signaling also down-regulates Cav2 and up-regulates Cav1 Ca^{2+} channel opening in acutely isolated MSNs [38, 42]. Furthermore, previous work has shown that the D_1-receptor-mediated enhancement of NMDA responses is dependent upon Cav1 channels [43]. The failure to detect a clear effect of D_1 agonists on bAP-evoked Ca^{2+} transients could be due to several experimental factors (e.g., disruption of intracellular signaling because of dialysis), but the most likely explanation is that this modulation is largely restricted to more distal, tertiary dendrites that were not effectively probed by a single bAP in D_1 MSNs.

In contrast to DA, it has generally been thought that acetylcholine (ACh), another potent modulator of MSN excitability released by giant aspiny interneurons with dense terminal fields overlapping those of DA neurons, affects both classes of MSN similarly. All MSNs robustly express M_1 muscarinic receptors [44]. The other muscarinic receptor expressed by MSNs, the M_4 receptor, is present in both classes, albeit at significantly higher levels in striatonigral D_1 MSNs [44]. Electrophysiological studies of muscarinic effects in randomly sampled MSNs have not reported pronounced heterogeneity [45–47]. However, more recent work with BAC transgenic mice has found a much stronger M_1-receptor-mediated modulation of dendritic Kir2 channels in striatopallidal D_2 MSNs than striatonigral D_1 MSNs, a difference attributable to the susceptibility of targeted channels, not upstream signaling [21] (see below). Muscarinic receptor agonists enhanced bAP-evoked dendritic Ca^{2+} transients in D_2 MSNs by local application of a muscarinic agonist but not in D_1 MSNs (Fig. 4.2). This modulation was occluded by 4-AP, suggesting that the modulation was mediated by M_1 receptor coupling to Kv4 channels, as found in pyramidal neurons [48, 49]. Why striatonigral D_1 MSNs should be unresponsive in spite of their expression of functional M_1 receptors and the dendritic localization of Kv4 channels is not clear.

ACh elevates MSN excitability by promoting closure of KCNQ (Kv7), SK and Kir2 K^+ channels [47, 50, 51]. Most, if not all, of these effects are attributable to M_1 muscarinic receptors, which are robustly expressed by both types of MSN [44, 46]. Although KCNQ channels are only active near spike threshold, Kir2 K^+ channels are constitutively active, serving to set resting

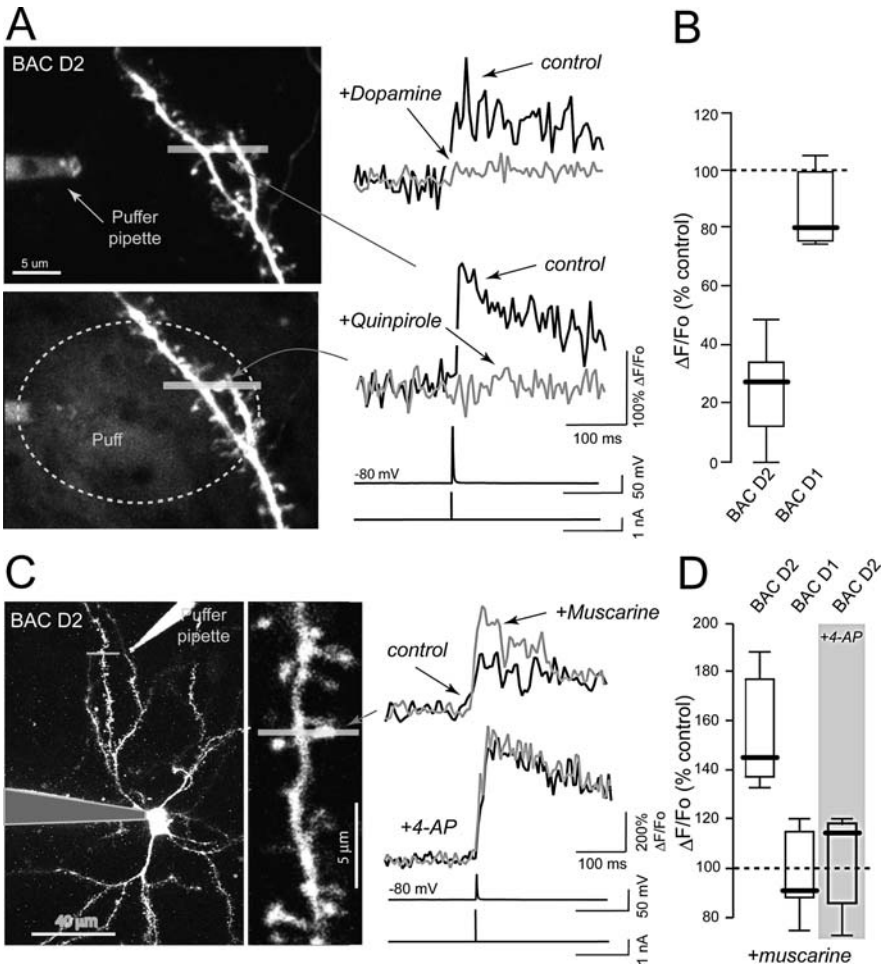

Fig. 4.2 Activation of D_2 receptors suppresses while activation of ACh receptors enhances bAP-evoked Ca^{2+} transients. (A) High magnification image of a D_2 MSN showing both the scan site (*gray line*) and the puffer pipette which contained Alexa Fluor 568 (10 μM) and quinpirole (10 μM) or dopamine (100 μM) for activating D_2 receptors. The traces show the bAP-evoked Ca^{2+} transient before (*black*) and during dopamine or quinpirole ejection (*gray*). **(B)** The *box plot* shows a significant quinpirole-induced reduction in the amplitude of the bAP-evoked Ca^{2+} transient in D_2 MSN spines; median = 27% of control (control, *dashed line*), $p < 0.01$, Kruskal–Wallis ANOVA, $n = 5$ cells. BAP-evoked Ca^{2+} transients in D_1 MSNs did not vary from controls taken before the dopamine puff ($n = 5$). **(C)** High magnification image of a D_2 MSN showing both the scan site (*gray line*) and the puffer pipette which contained Alexa Fluor 568 (10 μM) and muscarine (10 μM) for activating ACh receptors. The traces show the bAP-evoked Ca^{2+} transient before (*black*) and during muscarine ejection (*gray*). The *lower set of traces* in Fig. 4.3C show that the ACh-induced enhancement is occluded by 4-AP (1 mM in bath). **(D)** The *box plot* shows a significant enhancement in the amplitude of the bAP-evoked Ca^{2+} transient in D_2 MSN spines; median percent control = 145% (control, *dashed line*), Kruskal–Wallis ANOVA, $p < 0.01$, $n = 5$ cells. BAP-evoked Ca^{2+} transients in D_1 MSNs and in D_2 MSNs +4-AP did not vary from controls taken before the muscarine puff ($n = 5$ each)

membrane potential and dendritic input resistance – a key factor governing synaptic integration [52].

Strongly rectifying Kir2 channels are the principal determinants of the resting membrane potential and basal excitability of striatal MSNs [53, 54]. Neuronal Kir2 channels are multimeric transmembrane proteins constructed from a family of at least four subunits (Kir2.1–2.4) [55–58]. These subunits are expressed widely in the brain and in striatal MSNs [31, 53, 59]. Although primarily dependent on Kir2 channels, the K^+ channels active at the resting membrane potential are likely to include members of the KCNK class. Members of this class – KCNK2 (TREK-1) and KCNK10 (TREK-2) – that are expressed in the striatum give rise to a linear current–voltage relationship and are relatively resistant to Ba^{2+} block [60–62]. The currents evoked in MSNs by voltage steps or ramps could readily be broken down into a large, strongly rectifying Kir2 component and a smaller, linear KCNK-like component.

M_1 muscarinic receptor signaling potently down-regulated currents flowing through inwardly rectifying Kir2 channels in striatopallidal MSNs, but only weakly reduced currents in striatonigral MSNs. Although the M_1-receptor-mediated modulation of Kir2 channels in MSNs had been reported previously [47, 63, 64], the magnitude of the modulation and its cellular specificity have not been appreciated. The susceptibility of Kir2 channels in striatopallidal neurons to M_1 muscarinic receptor signaling did not appear to be due to differences in the transduction pathway itself. M_1 receptors are robustly expressed by both striatopallidal and striatonigral MSNs [44, 46] and their activation led to a very similar modulation of another PIP_2-dependent channel type – KCNQ (Kv7) K^+ channels. What did appear responsible was a difference in the molecular composition of the targeted channels. Although MSNs co-expressed readily detectable levels of mRNA for three Kir2 subunits – Kir2.1, Kir2.2 and Kir2.3, quantitative measures of mRNA abundance revealed that striatopallidal MSNs expressed roughly twofold more Kir2.3 mRNA than striatonigral MSNs. This was of functional importance because Kir2.3 subunits have a relatively low PIP_2 affinity, making them more susceptible than other subunits to changes in local PIP_2 levels induced by GPCR activation of PLC [65, 66]. Although these subunits are capable of forming heteromeric channels [67], our immunocytochemical assays suggest that Kir2.1- and Kir2.3-containing channels are not extensively co-localized. The potency of the M_1 receptor modulation of these currents (40–50% reduction) is close to what would be predicted from this observation and the relative abundance of Kir2.3 subunits.

In other neurons, Kir2 channels are enriched in dendritic regions [31]. Localization of Kir2 subunits has been difficult in MSNs because of a dense, irregular striatal neuropil. The use of a corticostriatal co-culture preparation [68] allowed us to overcome this obstacle and revealed not only that Kir2 channels were dendritic but that localization was subunit specific. Kir2.1 subunits were found largely in patches along dendritic shafts, whereas Kir2.3 subunits were largely restricted to dendritic spines upon which glutamatergic synapses are formed. Perisynaptic positioning of Kir2.3 subunits has also been seen in other forebrain neurons, where it is maintained by a PDZ domain

interaction with MAGUK family proteins [69, 70]. Although this scaffolding interaction is not unique to Kir2.3 subunits (both Kir2.1 and Kir2.2 are capable of binding to MAGUK proteins [71]) only these subunits were consistently found in MSN spine heads. M_1 muscarinic receptors are also localized to spine heads in MSNs [46] through an as yet undefined anchoring mechanism. Scaffolding was critical to the modulation of Kir2 channels by M_1 receptors, as it was significantly attenuated by disruption of a Kir2.3-PDZ motif mime (ESRI). Thus, bringing M_1 receptors (and presumably PLC) into close physical proximity with Kir2.3 channels appears to create an effective signaling microdomain [72].

The M_1-receptor-mediated modulation of Kir2 channels significantly enhanced the temporal summation of glutamatergic EPSPs. These glutamatergic EPSPs arise primarily from synapses formed on spine heads [22]. Although Kir2.3 channels were positioned near these spinous synapses, it is not clear that this positioning was important for enhancing summation (in contrast to signaling). Several lines of study suggest that spines do not act as electrical compartments [73, 74]. Nevertheless, diminished Kir2 channel opening within a region of dendrite should not only enhance temporal summation of EPSPs but their spatial summation as well; in so doing, the M_1 receptor modulation should promote the transition to depolarized 'up-states' and the opening of NMDA and Cav1.3 Ca^{2+} channels.

Disconnection of the Indirect Pathway in PD Models

Within days, DA depletion triggers a profound loss of spines and synapses in D_2 receptor expressing striatopallidal MSNs, without demonstrably affecting spine density in neighboring striatonigral MSNs. This rapid pruning appears to be part of a homeostatic response to elevated synaptic efficacy as it is prevented by genetic deletion (or blockade) of synaptically positioned Cav1.3 Ca^{2+} channels or the elimination of glutamatergic input [20, 75]. Although the loss of spines and glutamatergic synapses following dopamine depletion was consistent with previous studies in animal models of Parkinson's disease and in Parkinson's disease patients [76–80], the speed, selectivity and magnitude of the loss were not appreciated by these studies. In parallel with the elimination of glutamatergic synaptic contacts, the dendritic trees of striatopallidal neurons shrank, suggesting that the overall loss in glutamatergic synaptic input must be even more profound. The extent of the loss did not appear to be significantly different 1 month following dopamine depletion with 6-OHDA, suggesting that the regulatory processes controlling synapse elimination are complete within days and dependent upon the loss of dopamine, not the death of dopaminergic neurons.

The mechanisms underlying cellular specificity of the response are not completely understood but several factors appear to be involved. One major element in the cascade of events leading to pruning is the Cav1.3 L-type Ca^{2+} channel. These channels are dendritically positioned through a scaffolding interaction with Shank [81]. In acutely isolated MSNs, D_2 receptor signaling

decreases the open probability of Cav1.3 channels [37, 38]. Our results suggest that this modulation is also present in dendrites, showing that dendritic D_2 receptors decrease bAP-evoked Ca^{2+} transients in D_2 MSNs. Genetically deleting these channels or antagonizing them with systemically administered dihydropyridines dramatically reduced spine pruning following DA depletion, arguing that their opening and the Ca^{2+} influx triggered by opening was a key step in triggering synaptic pruning. The molecular mechanisms linking Cav1.3 channel Ca^{2+} flux and synaptic pruning remain to be explored. However, sustained elevations in intraspine Ca^{2+} can trigger disassembly of the cytoskeleton supporting spine morphology [82, 83] and L-type Ca^{2+} channels are linked to signaling cascades implicated in synaptic plasticity and alterations in transcriptional activity [81, 84–86].

Are there other factors (besides the loss of inhibitory D_2 receptor signaling) that might contribute to increased Cav1.3 channel opening following DA depletion? A second explanation that DA depletion facilitates an increase in ACh tone has been long hypothesized to underlie some of the disorders seen in patients with PD. In D_2 MSNs, blocking ACh suppressed the bAP-evoked Ca^{2+} transient in the DA-depleted mice as compared to untreated controls (Fig. 4.3). This suggests that cholinergic tone is elevated in the DA-depleted mice leading to a down-regulation of Kv4 channels. The down-regulation of Kv4 channels could be sufficient to enhance dendritic excitability in the DA-depleted D_2 MSNs or it could synergize with the decrease in spine density to render the dendrites even more excitable.

As mentioned above, M_1 muscarinic receptor signaling down-regulates dendritic Kir2 channels in striatopallidal D_2 MSNs, increasing the dendritic depolarization produced by glutamatergic synapses. M_1 receptor signaling also down-regulates dendritic Kv4 channels, increasing bAP invasion into distal dendrites. The importance of these M_1-receptor-mediated effects is underscored by attenuation of dendritic remodeling in M_1 receptor knockout mice following DA depletion [21]. In vivo, where M_1 receptor tone is undoubtedly higher than in the slice, D_2 MSN dendrites could be even more excitable following DA depletion. This combination of these M_1 receptor-signaling effects and the loss of inhibitory D_2 receptor control of Cav1.3 Ca^{2+} channels could be the essential dendritic events leading to synaptic pruning seen in D_2 MSNs of PD models.

In animal models of Parkinson's disease, short-term synaptic integration and dendritic morphology appear to be altered, at least in some medium spiny neurons [6, 8, 76, 77, 87–89]. The loss of D_2 receptor stimulation will also handicap the induction of LTD that might serve to normalize global activity without eliminating synapses [90–92]. Recent work by our group [93] has revealed that the loss of D_2 receptor stimulation not only prevents the induction of LTD in D_2 MSNs but it also promotes LTP induction through adenosine A2a receptor-signaling mechanisms. This interaction is mediated by an antagonism between the signaling mechanisms promoting LTD (D_2 receptor dependent) and those promoting LTP (A2a receptor dependent). The loss of D_2 receptor signaling disrupts the balance between these two processes, leading to strengthening of synaptic connections in inappropriate situations. This

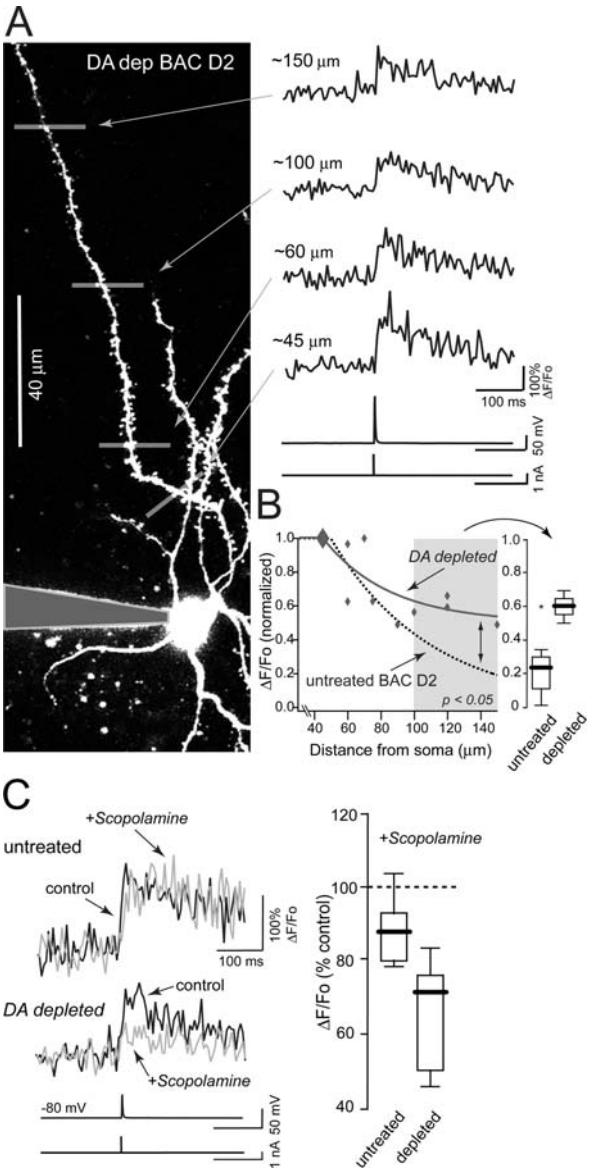

Fig. 4.3 DA depletion enhances excitability in distal dendrites in D $_2$ MSNs. (**A**) Maximum projection image of a D_2 MSN soma and dendrite from a DA-depleted BAC D_2 mouse (*left*). The *traces* show the bAP-evoked Ca^{2+} transient recorded at four different eccentricities along this dendrite (45, 60, 100, 150 µm, *right*). (**B**) Plot of the amplitude of the bAP-evoked Ca^{2+} transient normalized to the most proximal recording in each cell (*gray diamonds, line*). For comparison, the fit line from the D_2 untreated MSNs (Fig. 4.1D, *dashed line*) is added to the plot. The *box plot* demonstrates the increase in the amplitude of the normalized bAP-evoked Ca^{2+} in the distal regions of the DA-depleted D_2 MSN dendrites compared to control (untreated D_2 = 0.24, n = 4; DA-depleted D_2 = 0.6, n = 4; Kruskal–Wallis ANOVA, $p < 0.05$).

maladaptive response to DA depletion together with the elevation in dendritic excitability attributable to down-regulation of Kir2 and Kv4 K^+ channels might provide an explanation for the anomalous increase in glutamatergic mEPSC frequency seen in several studies of MSNs in PD models [7, 87, 94].

Another potentially important factor in the elevation of dendritic excitability and synaptic pruning is the loss of spines themselves. Spines constitute a large fraction of the dendritic surface area in MSNs [52]. The elimination of spines without a corresponding increase in dendritic diameter will decrease dendritic capacitance and enhance the ability of passively propagated APs to invade the dendrites. Simulations show that reducing dendritic spine density by 50% is sufficient to reproduce the elevation in bAP-induced Ca^{2+} entry in D_2 MSNs following DA depletion [25]. If this Ca^{2+} entry is an important factor in pruning, then spine loss should beget more spine loss without some other form of compensation.

Although the majority of the glutamatergic synapses formed on dendritic spines are of cortical origin, many are not [95]. The thalamic innervation of MSNs is similar in magnitude to that of the cerebral cortex, perhaps constituting as much as 40% of the total glutamatergic input to MSNs, terminating on both shafts and spines. Anatomical studies suggest that the intralaminar nuclei target primarily striatonigral neurons in primate striatum; however, this might not be the case in rodents [96], whereas 'motor' nuclei (VA, VL) project primarily to striatopallidal neurons [97, 98]. This apparent dichotomy between motor and 'associative' inputs is consistent with recent studies suggesting that input to striatopallidal neurons comes largely from pyramidal neurons contributing to descending motor control circuits, whereas the input to striatonigral neurons comes from neurons whose axons are largely intra-telencephalic [99]. Thus, it would seem that the regions of the brain most directly linked to motor functions become disconnected from the so-called indirect pathway, while the most parsimonious hypothesis is that it is primarily the cortical projection that is cut, this has yet to be rigorously tested.

Functional Implications for the Pathophysiology in PD

Several lines of evidence point to the importance of striatopallidal neurons in the expression of Parkinson's disease motor symptoms [13, 100]. Perhaps the most compelling of these is the finding that the activity of neurons in structures

Fig 4.3 (continued) (**C**) The *upper traces* show the bAP-evoked Ca^{2+} transient in a distal dendrite from an untreated BAC D_2 mouse before (*black*) and during bath application of 20 μM scopolamine (*gray*) to block tonic ACh. The *lower traces* were taken from a distal dendrite in a DA-depleted BAC D_2 mouse before (*black*) and during bath application of 20 μM scopolamine (*gray*). The *box plot* shows that scopolamine suppresses bAP-evoked Ca^{2+} transients in the DA-depleted mice as compared to untreated controls with the median % of control decreasing from 87% in untreated mice to 71% in the DA-depleted mice ($n = 5$ cells each; Kruskal–Wallis ANOVA, $p < 0.05$)

controlled by striatopallidal neurons is dramatically altered in people suffering from Parkinson's disease and in animal models of the disease. Neurons in the globus pallidus and in the reciprocally connected subthalamic nucleus begin to discharge in anomalous rhythmic bursts that are often synchronized. Silencing this abnormal patterning with lesions or deep brain stimulation provides dramatic relief from motor symptoms [101, 102]. Computer simulations grounded in experimental observation suggest that this rhythmic bursting is an intrinsic property of the pallido-subthalamic circuitry that is normally suppressed by striatopallidal GABAergic inhibition [103]. Ineffectively timed or patterned striatopallidal activity could 'release' this circuitry, allowing it to display activity patterns like those seen in Parkinson's disease. Because striatopallidal medium spiny neurons depend upon highly convergent glutamatergic synaptic inputs from cortical and thalamic motor command centers [104], the loss of a substantial portion of this input should profoundly disrupt movement related, patterned activity and in so doing limit their ability to control the emergence of synchronous bursting in the pallido-subthalamic circuit. The failure to control the pallido-subthalamic circuit should lead to unwanted movements and the cardinal symptom of Parkinson's disease – the inability to translate thought into efficient movement.

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Chapter 5
Diversity of Up-State Voltage Transitions During Different Network States

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Introduction

Information processing through a neuronal microcircuit depends essentially on three major features: (1) the anatomical organization and the function of its synaptic connections; (2) the biophysical or intrinsic properties of its neuronal elements; and (3) how the synaptic and biophysical functions are modified by the modulatory transmitters acting via intracellular signaling cascades.

The whole understanding of these processes requires an integration of knowledge ranging from molecular biology to computational neuroscience, passing through cellular and systems neurophysiology. Nonetheless, two identifiable key properties have been recognized of general importance for the functioning of microcircuits: the variety of neuronal classes as defined by their ability to exhibit diverse firing patterns depending on their intrinsic ionic conductances and their modulation [1], and the arrangement of connections between neurons to form neuronal structures or networks capable of pattern generation, the propagation of correlated and synchronized firing, bistability, and computational powers such as memory storage, retrieval and compositionality [2, 3]. In the present chapter we review the dependency of striatal circuit dynamics on a general property of spiny neurons: their ability to exhibit voltage transitions of their membrane potential between a hyperpolarized, mostly inactive, "down"-state and a depolarized, commonly bursting, "up"-state.

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Striatal Circuit Arrangement

Medium spiny neurons (MSNs) are the projection neurons of the striatum. In the rodent, they represent 90–95 % of its neuronal population. These GABAergic neurons receive their name from the large number of spines that cover their dendrites. Their cell body ranges from 10 to 20 μm in diameter, surrounded by a spherical dendritic tree with a radius of about 250 μm [4]. The main afferents bringing fast ligand-gated synaptic transmission to MSNs come from the cortex and the thalamus. These afferents make glutamatergic excitatory synapses on the heads of the dendritic spines [4]. Corticostriatal and thalamostriatal connections reveal a sparse arborization pattern invading the striatum to activate neurons in a widespread area [5, 6]. A unique cortical or thalamic neuron makes a few, *en passant* synapses, with any given MSN. That is, MSNs need numerous synchronous inputs from a number of cortical or thalamic afferent fibers to generate a train of action potentials. These afferents come from the whole cortical mantle and, accordingly, behavioral studies show that the striatal network can work as a matrix for associative learning and storage of procedural memories and habits [7]. Thus, the activity of a given MSN may be controlled by neurons from wide apart cortical areas and not only by a few cortical or thalamic neurons. Additionally, this may implicate that nearby MSNs do not share the same cortical or thalamic inputs, while neurons far away from each other may do [8]. In other words, neurons responding to the same inputs, probably belonging to the same neuronal ensemble, are intermingled with neurons from other ensembles, as it happens in layers II–III of the neocortex [9–11].

Most inhibitory inputs onto MSNs come from inside the nucleus: two-thirds from other MSNs and one-third from interneurons [12]. Electrophysiologically, there are two basic interneuron genres: the parvalbumin immunoreactive fast-spiking (FS) interneurons and the somatostatin-containing low threshold spiking (LTS) interneurons [13]. However, there are many more types in between these two classes.

Individually, synaptic connections between MSNs are weaker than those made between interneurons and MSNs: [13] each MSN only makes a few contacts with other MSNs, while an interneuron makes larger and more numerous contacts with more MSNs. Therefore, the inhibitory synaptic arrangement forces a synchronized convergent firing of many MSNs to produce an inhibition as strong as that produced by a single or a few interneurons, indicating that both types of inhibition have different purposes. However, the fact that, intrinsically, the striatum is an inhibitory interconnected network explains in part why this nucleus is considered an almost “silent” nucleus, with only a few cells having activity at a given moment.

These synaptic arrangements suggest that information processing in the striatal network needs correlated firing: convergent synchronized firing is needed for most excitatory and inhibitory inputs to produce significant synaptic actions on MSNs.

The Bistable-Like Behavior of Striatal Medium Spiny Neurons

One significant synaptic action recorded in MSNs is their bistable-like behavior [14]. It is characterized by voltage transitions of the membrane potential between two preferred states: (i) a quiescent highly polarized potential or "down"-state, negative to the reversal potential for inhibitory postsynaptic potentials (IPSPs), which is near –65 mV, and near to the potassium equilibrium potential which is near –80 mV and (ii) a depolarized level or "up"-state, in which synaptic potentials of only a few millivolts can reach spike threshold and generate action potential discharge [14]. Active MSNs are spontaneously switching between these two states.

The "down"-state is dominated by powerful inwardly rectifying potassium conductances (gKir) that account for a low input resistance and time constant at rest [15, 16]. These conductances are responsible for the low intrinsic excitability of these cells and constitute the other main reason why the striatum is known as a "silent" nucleus.

To summarize, the striatal circuitry design is such that weak and uncorrelated synaptic inputs may not be able to activate MSNs. But correlated and convergent synaptic inputs produce a shift from the down-state to the up-state transforming the neuron from inactive to active. Synchronous inputs depolarize the cell and close gKir [14, 17, 18]. Another way to see this matter is considering the postsynaptic neuron as a coincidence detector [19]. The spiny neuron would be a filter of barrages of synaptic inputs, allowing transmission of relevant information only: coherent activation of behaviorally significant cortical and/or thalamic neuronal ensembles.

However, once the conditions for an appropriate level of depolarization have been met, several events occur in sequence in MSNs: (i) gKir channels close, enhancing input resistance and lowering the electrotonic distance of the dendritic arbor; (ii) gKir closing suddenly increases the strength of synaptic inputs in the dendrites and recruit N-methyl-D-aspartate (NMDA) synaptic channel receptors; (iii) temporal and spatial integration of these inputs is consequently enhanced and prolonged so that (iv) the level of depolarization may reach the activation voltage of several voltage-dependent ionic conductances such as (a) Ca^{2+}-channels of the $Ca_V1.3$ class whose activation voltage is near the threshold for firing action potentials [17, 20, 21], (b) slow Na^+ and or cationic conductances [22–24], and (c) a diverse array of K^+-conductances, some transitory or inactivating as the $K_V1.2$ K^+-channels (I_D) [25–27] and some persistent and slowly activating as the KCNQ (I_M) channels [28]. NMDA, Ca^{2+} and Na^+ currents would create a negative slope conductance region conveying bistability and non-linear dynamics [29] and conferring the ability to have more sustained and stable up-states [18], while outward currents would limit the level of depolarization of these up-states [30], leaving the voltage in a range where certain patterns of synaptic inputs become capable to generate bursts of spikes. These events involve the interaction between synaptic inputs and intrinsic

properties. The question arises about how stereotyped or constant this sequence of events is, that is, if every time up-states are made up with the same mixture of synaptic and intrinsic conductances or if there is a place for variability.

Variability may be the action of the third factor in the workings of a circuit: the activation of intracellular signaling cascades by modulatory neurotransmitters. It has been demonstrated that they play a role. To solely talk about the most important modulatory neurotransmitters in the striatum, acetylcholine (ACh) and dopamine (DA), numerous studies have demonstrated that their signaling modifies many of the factors listed above. Thus, both ACh and DA modify the AMPA, NMDA, Ca^{2+} and Na^{2+} conductances, that is, the whole array of voltage-activated and ligand-activated inward currents that may bring up bistable properties [17, 22, 31–39]. Second, outward currents have also been shown to be modulated [28], together with inward-rectifying K^+ conductances [24, 32, 38] and GABAergic inhibitory synapses [40].

In other words, because brain states (e.g., vigilance, wakefulness, slow wave sleep, REM sleep, or anesthesia) bring about different mixtures of these (and other) modulators into the extracellular space, and because these modulators are known to modify intrinsic and synaptic conductances, it would be strange to expect the same stereotyped voltage transitions during different brain states. Given that in order to be activated MSNs need this sort of synchronized synaptic inputs during any brain state, the following evidence, some of it unpublished, intends to give a hint about the enormous variability, waiting to be systematically studied, present during voltage transitions in MSNs, and probably, in many other neuronal classes.

Up and Down Voltage Transitions in MSNs

MSNs remain in the quiescent down-state in the anesthetized animal under neuroleptic analgesia [41] or in animals with cortical deactivation [14]. Voltage transitions of MSNs do not spontaneously occur in the in vitro slice preparation, although they can be triggered by external stimulus [18]. It is therefore likely that the most commonly recorded voltage transitions in vivo need a large barrage of excitatory inputs and correlated firing [14]. In fact, synaptic conductances are higher throughout the up-state and corticostriatal neurons registered on the ketamine–xylazine anesthetized preparation show state transitions similar to those of striatal neurons [5]. Thus, synchronous cortical synaptic inputs to the MSNs, balanced by voltage-activated K^+ conductances, make up the up-state observed on the ketamine–xylazine anesthetized rat preparation.

Nevertheless, intracellular recordings of the MSNs activity in the head-restrained non-anesthetized rat [42] have shown that membrane potential transitions largely depend on the vigilance state of the animal (brain states) and on the degree of synchronous activity of the cerebral cortex (Fig. 5.1). As expected from previous work [41, 43–45], it was confirmed that the bistable-like behavior

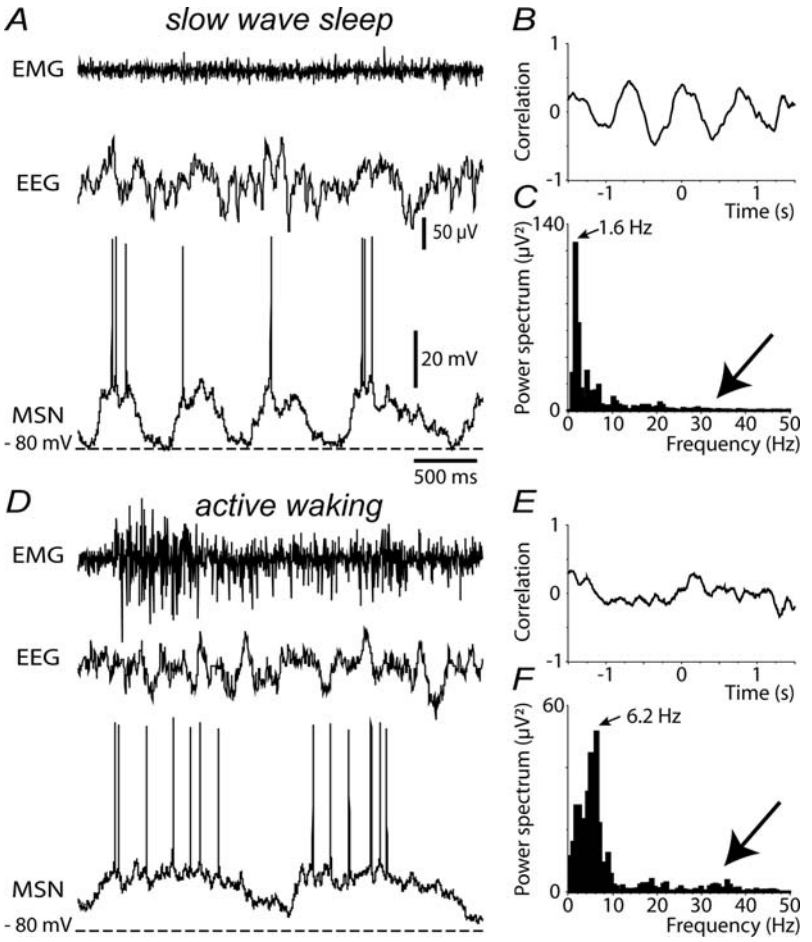

Fig. 5.1 "Up-states" in vivo. (A) Intracellular recording from a neostriatal MSN during slow wave sleep. During this brain state, MSNs activity is characterized by relatively abrupt membrane potential transitions between quiescent hyperpolarized down-states and depolarized up-states associated with firing, similar to those described in the ketamine–xylazine anesthetized rat preparation [41, 46]. SWS is characterized by high-amplitude low-frequency electroencephalographic waves (EEG) and a weak muscular activity (EMG). (B) EEG and intracellular subthreshold activities present a strong periodic correlation consistent with the dominant frequency encountered in the EEG waves (~1–2 Hz) and revealed by their power spectrum. (C) *Arrow* shows the absence of high-frequency rhythms during this brain state. (D) Intracellular activity from a neostriatal MSN during active waking. During this brain state, animal movements observed in the electromyogram (EMG) can be associated with a slowly developing membrane potential transition to a depolarized state followed by a long-lasting plateau potential. Potentials similar to these have also been called "up-states" in the current literature. This activity is concomitant to low-amplitude high-frequency EEG waves. (E) A relatively flat cross-correlogram shows that massive synaptic activity from the cortex is not necessary to see these "up-states" or "plateau potentials". (F) Spectral analysis shows that the EEG activity associated with active waking is dominated by theta-frequency oscillations (~5–9 Hz) while high-frequency oscillations (beta >20 and gamma 30> Hz) are also present (*arrow*)

of MSNs described on the ketamine–xylazine anesthetized rat preparation does not reflect the spontaneous activity of the spiny neurons during wakefulness but more likely reproduce the dynamics of cortical activity during SWS (ketamine is itself a blocker of NMDA receptors) (Fig. 5.1A–C) [42].

However, as illustrated in Fig. 5.1D–F, up-state voltage transitions can also be observed during episodes of active waking and more importantly, in relation to movements of the animal (N. Vautrelle, unpublished). Interestingly, such movement-related "up-states", which are also called "plateau potentials" in many brain nuclei, closely resemble those observed in vitro after bath application of NMDA (Fig. 5.2) and are not correlated to a sustained synchronized activity of the cortex (Fig. 5.1D–F), although a better analysis is necessary to discard fleeting or discrete synchrony in the EEG activity. Bursting and trains of action potentials in MSNs in relation to movement have been recorded for a long time in the striatum, extracellularly [47–49], it is the subthreshold behavior of the membrane potential during these episodes that had not been reported.

Two-state transitions of MSNs membrane potential can also be induced in the striatal brain slice preparation maintained in vitro by bath application of NMDA, an agonist of glutamate receptors (Fig. 5.2A) [10, 18]. These transitions can be generated even if cortical and thalamic afferent inputs are removed; however, they may be more easily generated if NMDA applications are accompanied by cortical stimulation [18]. Moreover, comparison of "up-states" induced in vitro by NMDA with "up-states" recorded in vivo in relation to movements shows several similarities (Fig. 5.2C): they arise from more depolarized membrane potential (down-state: –70 or –75 mV) than those recorded during sleep or anesthesia ($\leq$ –80 mV), and they have similar time courses during the transitions from the down-state to the up-state and vice versa (N. Vautrelle, unpublished).

Voltage transitions (plateau potentials or up-states) induced in vitro with NMDA and those related with movement and recorded in vivo are indeed very similar, and at the same time, very different to those recorded during sleep or anesthesia. This finding indicates, first, that there are different types of "up-states" that although they look similar at a first glance will readily show their differences on a closer observation. No one knows how many varieties of up-states or voltage transitions there are. Second, it is also shown that up-states can be generated in MSNs independently of a massive excitatory synaptic barrage. Finally, it is shown that up-states can be seen in the context of specific sparse circuitry activation. This may not seem surprising because, previously, it has been shown that NMDA administered in vivo unilaterally in one striatum induces movements and turning behavior [50]. Moreover, NMDA has long been used in the spinal chord, brain stem and diverse cerebral nuclei, including the cortex, to set into action local circuits and movement-related "central pattern generators" (CPGs) [3, 51–53].

As illustrated in Fig. 5.2B, the two-state behavior of MSNs induced in vitro by bath application of NMDA is associated with the emergence of a negative slope conductance region in the current–voltage relationship measured in

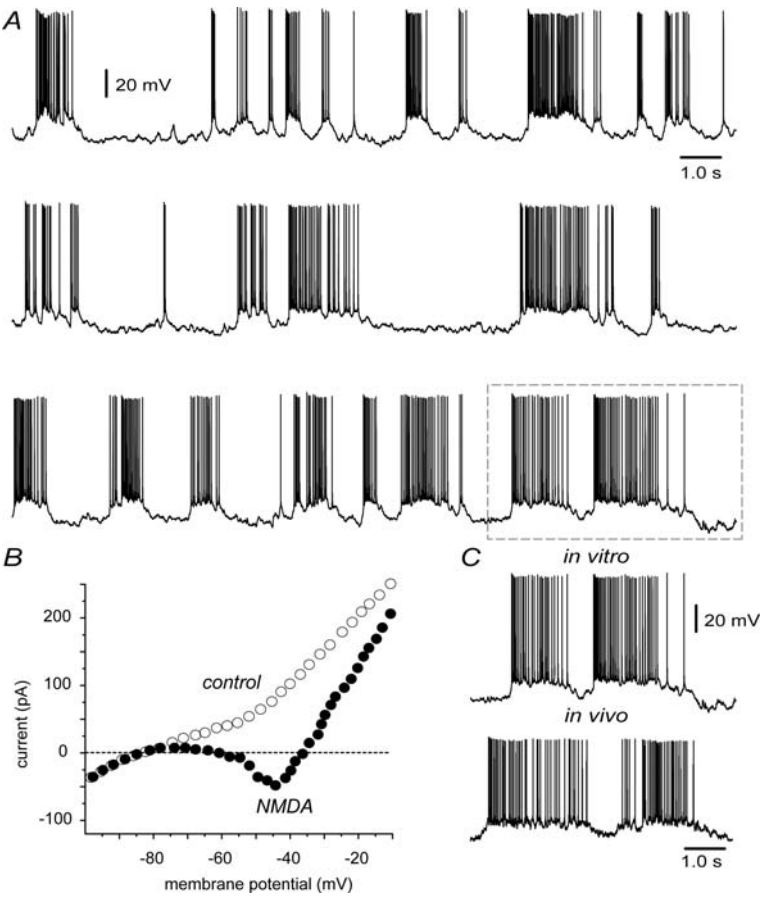

Fig. 5.2 Up-states in vitro and in vivo. (A) The three rows show a continuous recording from a neostriatal medium spiny neuron identified both electrophysiologically and after biocytin histochemistry (not shown). Note that spontaneous activity (without electrical stimulation of its afferent inputs) is consistent with recurrent voltage transitions from a down-state (silent) to an up-state (bursting). This activity was recorded in vitro. It can be induced with bath addition of NMDA (5–10 μM) and facilitated with cortical stimulation in corticostriatal brain slice preparations [18]. (B) Current–voltage relationships from medium spiny neostriatal neurons in control (*open circles*: note a single crossing on the voltage axis – *dashed line*), and in the bath presence of NMDA (*filled circles*: note a negative slope conductance region – NSCR – and three crossing points on the voltage axis, a landmark of bistability and non-linear capabilities). (C) Two up-states recorded in vitro (*top*: from *dashed square* in (A)) compared with two up-states recorded in vivo during active waking with movement (note similarities and compare with up-states from sleep in Fig. 5.1A)

voltage clamp [10, 18]. This negative slope conductance region (NSCR) is the landmark of bistability and non-linear dynamical properties [29, 54]. In the case of MSNs, the NSCR induced by NMDA is sensitive to the application of Cd^{2+} and is abolished by the application of dihydropyridines, blockers of the Ca_V1 (or L-type) Ca^{2+} channels [18].

To conclude, up and down voltage transitions of MSNs do not always reflect simple massive conduction from overlying cortex. MSNs seem to be able to generate not only one but several kinds of bistable-like behavior (unpublished data) which greatly depend on the brain state of the animal, which in turn depends on the state of modulation in the circuit. Inasmuch as brain states depend on neuromodulatory transmitters, the same neuronal elements of a network may produce a diverse output activity, enhancing then the functional complexity of neuronal microcircuits.

Up-States: Windows for Ensemble Synchronization

If in order to elicit up-states there is a need for synchronized firing, the elements synchronized may differ depending on the brain state. If up-states during sleep and anesthesia are mostly synaptic [55, 56], it is because a great number of elements are driven by slow oscillations involving large areas of the cortex and thalamo-cortical loops [41, 43, 57]. This is a global code. Intrinsic conductances even if activated may be shunted by the large amount of synaptic conductances.

However, during movement, in the awake animal, it is possible that a select group of neurons in dedicated circuits are the ones activated in a sort of a sparse code. Although synchronization of specific elements is still necessary in order to produce a depolarization level that generates action potential firing [10], intrinsic conductances will now boost the actions of synaptic ones. This may produce a "window of synchronization" for the different elements of an ensemble [58]. A test of this hypothesis is shown in Fig. 5.3: striatal neuronal ensembles exhibit spontaneous peaks of synchronous activity during NMDA [10]. However, peaks of synchronization are lost after addition of dihydropyridines, blockers of L-type Ca^{2+}-channels (Fig. 5.3A). Electrophysiological recordings of ensemble elements can be shown to be mainly medium spiny neurons (Fig. 5.3B). Moreover, in the case of MSNs, the NSCR is sensitive to the application of Cd^{2+} and is abolished by the application of dihydropyridines, (Fig. 5.3C). These results indicate that the activation of L-type Ca^{2+} channels, expressed in the dendrites at the site of corticostriatal synapses of MSNs [21], opens a temporal window which can lengthen plateau potentials capable to sustain repetitive firing and substitute for a larger barrage of synaptic inputs coming from the cortex as seen during SWS.

Conclusions

"Up-state" is a generic name for a commonly observed phenomenon, that is, the generation of sustained plateau-like depolarizations for the synaptic inputs to be capable to reach firing threshold and thus produce a train of action potentials. By looking at the literature in different brain nuclei, including the cortex and the spinal chord, it is easy to see that the shape and duration, the time course for reaching firing threshold, and the time course for leaving it and returning to the

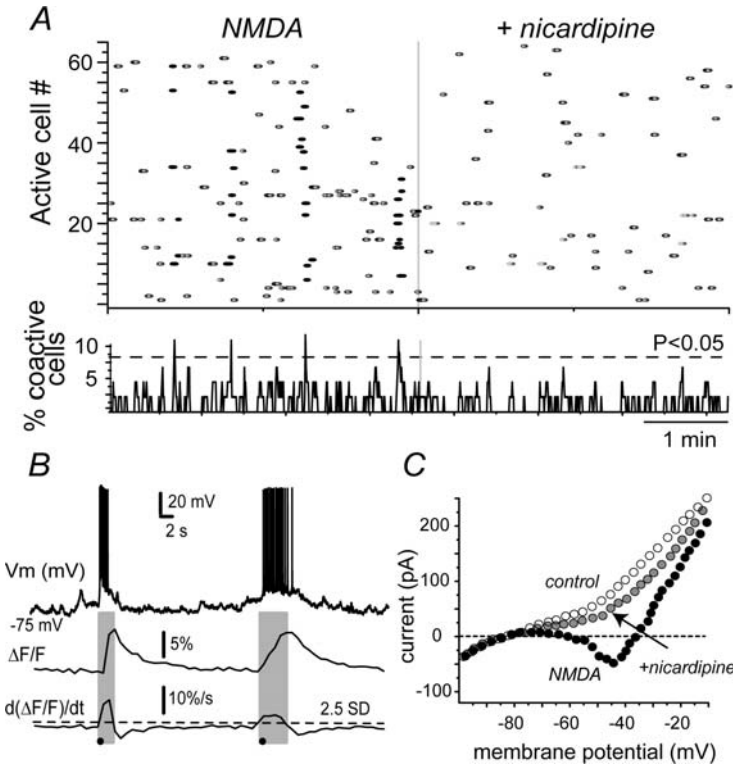

Fig. 5.3 Importance of calcium channels in network dynamics. (A) The raster plot of network activity illustrating the dynamics in the bath presence of NMDA (*left*) and after NMDA + nicardipine (*right*). Peaks of synchrony (*asterisks*) were abolished after nicardipine application even when activity was not much reduced. Histogram represents the percentage of coactive cells over time in the same experiment.
(B) Simultaneous recordings of voltage transitions (*top*) and calcium transients (*middle*) induced by the presence of NMDA in the bath from a medium spiny neuron. Note that the duration of the first derivative of the calcium transients match the duration of electrophysiological up-states. *Dots* indicate events used to reconstruct raster plots. (C) Current–voltage relationships from medium spiny neostriatal neurons after adding nicardipine, a dihydropyridine blocker of Ca_V1 class (L-type) Ca^{2+}-channels (*gray-filled circles*: note that even though the NSCR was produced by NMDA it was blocked by reducing an intrinsic inward current, suggesting that synaptic currents induce intrinsic ones)

down-state are highly variable for all the events that have been called "up-states". While some of these events may be mostly or purely synaptically driven (e.g., [56]), others involve intrinsic conductances (e.g., [54]). The amount of mixture between synaptic and intrinsic conductances (inward and outward) in different modulatory states of the network, or in different brain states in vivo, has not been systematically studied. Thus, while some investigators assume that everything is synaptically generated, and even calculate the amount of excitatory and inhibitory conductances, others go on and check different types of intrinsic conductance blockers and describe the changes that these blockers produce.

As an example, persistent sodium currents are associated with different motor behaviors including respiratory rhythms [59], swallowing [60] and hypoglossal-related movements [61]. On the other hand, bistable properties mediated by calcium currents have been reported in cerebellar Purkinje cells [62, 63], substantia nigra pars reticulata neurons [64], spinal motoneurons [65], and neostriatal spiny neurons [10, 18], among others.

Relation of voltage transitions with network and brain states have also been documented. Thus, activation of the dopaminergic system is associated with reward processes and improvement of task execution [48], while activation of the cholinergic system is associated with memory processes [66]. Accordingly, dopamine reduces the persistent sodium current [22] and may increase or reduce calcium current in medium spiny neurons [17, 33], while muscarine decreases calcium currents in the same cells [36]. Thus, diverse functional states may involve specific ionic currents which are differentially modulated by neurotransmitters.

The hypotheses advanced in this chapter, subject to experimental test in different neurons and circuitries, are that, first, the same cells may generate different types of bistability and, therefore, exhibit different types of up-states or voltage transitions and, second, that these differences may rely on different mixtures of synaptic and intrinsic conductances, mixtures brought about by the blend of neuromodulatory transmitters in the extracellular space (e.g., [51, 67]). Experimental work describing neuromodulatory actions on both synaptic and intrinsic conductances, and voltage transitions during circuitry processing, is common day neurophysiology. However, it is now needed the convergence of these fields to explain more global modulatory actions onto the activity of the circuits.

Finally, synchronization and desynchronization dynamically adjust the network states, relating elements of the system with a widespread spatial distribution. Such processes could be a useful mechanism to improve task execution suppressing false targets. In contrast, Parkinson disease involves a hypersynchronization between different elements of the basal ganglia nuclei [68]. The change of the network dynamics is therefore related to different combinations of synaptic weights and membrane intrinsic properties, all of it regulated by modulatory transmitters. Therefore, the challenge is to reveal not only the network structure but how the latter maintains a functional level, that is, how parameter combinations are maintained to encode and perform specific tasks.

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Chapter 6
The Corticostriatal Pathway in Parkinson's Disease

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Introduction

The cortico-basal ganglia-thalamocortical loop participates in the regulation of motor movements and goal-directed behaviors [1–4]. Parkinson's disease (PD), as well as other neurodegenerative disorders that affect motor function, is associated with abnormal neurotransmission along this pathway [5–7]. In this chapter, we will examine how a reduction of dopamine availability in PD produces striatal synaptic plasticity. These striatal adaptations might be sufficient to produce bradykinesia in the dopamine-deficient state and motor dyskinesias following treatment. Although changes occur at several levels of the cortico-basal ganglia-thalamocortical loop, the focus of this chapter is on the corticostriatal synapse.

Corticostriatal Anatomy and Function

The dorsal (motor) striatal microcircuit is composed of parallel sets of glutamatergic corticostriatal projections that synapse onto striatal medium-sized spiny neurons (MSSNs) [8–10] (Fig. 6.1). MSSNs constitute 90–95% of all striatal neurons and utilize γ-aminobutyric acid (GABA) as their principal neurotransmitter [11]. Each MSSN may possess 10,000 dendritic spines that receive numerous and diffuse projections from almost all areas of the neocortex [12]. The striatal microcircuit also contains a number of modulatory components including dopaminergic projections from the substantia nigra pars compacta [13, 14] and a variety of large striatal interneurons, including tonically active interneurons (TANs) that release acetylcholine [15–19] and fast-spiking GABAergic interneurons [20]. The modulation of cortical signals by these neurotransmitters at the MSSN determines signal transmission along the direct or indirect striatal output pathways and is critical for regulated motor control [10].

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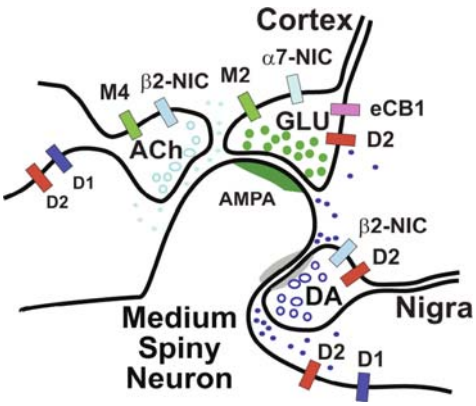

Fig. 6.1 In this simplified striatal microcircuit, dopaminergic (DA) nigrostriatal fibers and cholinergic (ACh) interneurons modulate excitatory glutamatergic (GLU) corticostriatal projections on medium spiny neurons. Neurotransmitter release is modified by D1 and D2 dopamine receptors, M2 and M4 muscarinic receptors and α7*- and β2*-nicotinic receptors and endocannabinoid (eCB1) receptors

Dopaminergic Projections and Receptors in the Motor Striatum

Dopamine is thought to play a crucial role in reinforcement and learning because it signals error in the prediction of future reward and switches attentional and behavioral selections to unexpected, behaviorally important stimuli. This switching response prepares the organism for an appropriate reaction to biological significant events [21]. Dopamine released from striatonigral projections binds to postsynaptic D1-like and D2-like receptors [22] that are distributed on MSSNs relatively far from the dopamine terminals, along the peripheral zone of synapses formed by glutamatergic terminals [23–26]. D2 autoreceptors are also located on presynaptic dopamine axon terminals distal from apparent active zones, where they provide a basis for dopamine autoregulation [27]. D2 receptors also exert their influence on a subset of corticostriatal projections [28] where they regulate glutamate release [29–34].

Besides their anatomic placement, dopamine receptors also differ in their binding affinity. D2 receptors exhibit nanomolar affinity for dopamine. Since synaptic dopamine concentrations associated with tonic nigrostriatal firing patterns reside at 5–20 nM, D2 receptors react to small changes in dopamine release [22, 35, 36]. D1 receptors exhibit a much lower (micromolar) affinity for dopamine than D2 receptors [22, 35, 36] and are activated by physiological burst activity or by dopamine-releasing psychostimulants such as amphetamine [37]. Following release, dopamine is subsequently cleared from the synaptic cleft through reuptake by the dopamine transporter located on the plasma membrane of dopaminergic terminals [38–40]. During tonic activity, dopamine

is completely cleared from the synaptic cleft by the dopamine transporter. During burst activity, however, the transporter is unable to rapidly clear dopamine. The resulting accumulation of dopamine is implicated in the acquisition of positively reinforced learning [41, 42]. Dopamine is not required for the formation of normal corticostriatal cytoarchitecture, glutamate density or basal striatal glutamate concentrations [30, 43], suggesting that dopamine is essential for movement, but is not required for the development of neural circuits that control those behaviors [44].

Striatal Organization

The striatum is organized into direct and indirect pathways that are determined by their striatal output projections and provide for the conversion of the excitatory striatal input into balanced opposed output systems [3, 45–47]. The striatal output is organized by segregation of separate populations of GABAergic MSSNs with distinct anatomic projections and neuropeptide expression [48]. Dopamine reinforces cortically initiated activation of a particular basal ganglia-thalamocortical circuit by facilitating conduction through the circuit's direct pathway, which has a net excitatory effect on the thalamus, and suppresses conduction through the indirect pathway, which has a net inhibitory effect on the thalamus [2]. The direct pathway promotes desired movements [1] while the indirect pathway inhibits movement by modulating disinhibitory drive [2].

The direct pathway consists of MSSNs that predominantly express D1 dopamine receptors [46], substance P [49] and dynorphin [50]. These neurons project to the substantia nigra pars reticulata (SNr) and to the globus pallidus interna (GPi; entopeduncular nucleus) [3, 45–47]. Direct pathway striatal neurons facilitate initiation of motor programs by eliciting a phasic inhibition of the GPi/SNr and by exerting tonic inhibitory control on the thalamus and brainstem motor areas [47]. The indirect pathway is comprised of striatal neurons that express predominantly D2 receptors [46], met-enkephalin and neurotensin [49, 51]. These neurons form projections to the external segment of the globus pallidus (GPe) [3, 48, 52]. Since the GPe tonically inhibits the substantia nigra, activation of the indirect striatopallidal pathway disinhibits the substantia nigra. The resulting increase in substantia nigra activity leads to an increase in inhibitory output from the GPi/SNr, resulting in inhibition or termination of motor programs. Dopaminergic inputs from the substantia nigra modulate the activity of these pathways, exerting a net excitatory effect on the direct pathway and a net inhibitory effect on the indirect pathway [47]. As D1 and D2 receptor-expressing MSSNs are largely segregated, the balanced opposition of these output systems is likely required to produce normal movements and motor learning.

Electrophysiological Properties of Striatal D1 and D2 Receptor-Containing MSSNs

Traditionally, it was believed that MSSNs giving rise to the direct and indirect pathways were morphologically and electrophysiologically identical. A major drawback of those earlier studies was that identification of MSSNs belonging to direct (D1 receptor-containing) or indirect (D2 receptor-containing) pathways was very difficult and the identity of the cell was only possible a posteriori. However, recent advances in neurogenetics have allowed the insertion of reporter genes, e.g., enhanced green fluorescent protein (EGFP), in the promoter region of D1 and D2 receptors permitting visualization and selection of striatal MSSNs containing these receptors before recordings are made. Although recordings in vitro demonstrate that most basic membrane properties and resting membrane potentials are similar in D1 and D2 receptor-containing MSSNs, important differences are beginning to emerge. For example, D2 cells are more excitable than D1 cells as they fire action potentials at more hyperpolarized membrane potentials [53]. Input–output curves also demonstrate that at similar current intensities, D2 cells fire more action potentials than D1 cells [54].

Differences in synaptic inputs have also been reported. Although initial studies examining spontaneous glutamatergic synaptic activity in D1 and D2 MSSNs did not find differences in the frequency of excitatory postsynaptic currents (EPSCs) [55], other studies have demonstrated significant dissimilarities. In standard cerebrospinal fluid, the frequency, of spontaneous EPSCs is higher in D2 cells compared to D1 cells and large-amplitude events are only seen in D2 cells [53]. After addition of the sodium channel blocker tetrodotoxin to isolate miniature EPSCs, the difference in the frequency of EPSCs between D1 and D2 cells is reduced but the cumulative inter-event interval distributions are still significantly different [53, 54]. Further, after addition of the $GABA_A$ receptor blockers bicuculline or picrotoxin, which induce epileptiform activity in cortical pyramidal neurons, D2 cells display large membrane depolarizations and inward currents rarely seen in D1 cells [53]. These effects imply that D2 receptor-containing MSSNs reflect cortical activity more reliably than D1 cells and support anatomical evidence indicating that the size of corticostriatal terminals making synaptic contacts with D2-immunolabeled spines is significantly larger than those making contact with D1-immunolabeled spines [56]. A possible functional consequence of this differential innervation could be that D2 cells are subject to increased action potential-dependent glutamate release from corticostriatal terminals. Interestingly, cortical pyramidal neurons innervating the indirect pathway (probably D2 cells) also receive more excitatory inputs due to enlarged dendritic trees in cortical layer I compared to pyramidal neurons innervating the direct pathway (probably D1 cells) [57]. The preferential propagation of epileptiform activity onto D2 cells also supports previous data demonstrating that enkephalin-positive neurons, that also express D2 receptors, are selectively activated by cortical stimulation [58]. Overall, these data

indicate that, compared to D1 cells, D2 cells appear to reflect more faithfully the on-going cortical activity, particularly the activity generated by pyramidal tract-type cortical neurons that preferentially innervate D2 cells [56].

There is also anatomical evidence for differential thalamo-striatal innervation [59]. Thalamic projections preferentially target striatal cholinergic interneurons and those from the centromedian nucleus form synapses preferentially with direct pathway MSSNs [60]. Understanding the informational content and value of different cortical and thalamic inputs and the way different subpopulations of MSSNs process those inputs will further our knowledge of striatal functions in physiological and pathological states.

Mouse Models for Parkinsonism

A variety of mouse models have been used to study the effects of dopamine deficiency on corticostriatal activity. Observed effects of dopamine depletion including mutant mice in which dopamine production is deficient [44], the synaptic vesicle catecholamine depleting agent reserpine, and lesioning experiments using catecholamine neurotoxins [61] have all confirmed the specific role of dopamine in motor control. Reserpine-treated mice [62] are used to investigate the effects of acute dopamine depletion [30]. Reserpine produces a rapid decrease in brain dopamine concentration with a reduction to $<1\%$, 13 h following treatment [30]. Although these models have been invaluable to elucidate the consequences of the loss of dopaminergic neurons and develop new symptomatic therapies, their usefulness to study the pathophysiology of PD remains limited because they are mostly based on neurotoxic mechanisms.

Dopamine-deficient mice are used to model the effects of chronic dopamine deficiency. Dopamine-depleted mice were generated by a targeted deletion of the tyrosine hydroxylase (*Th*) gene in dopamine neurons while restoring *Th* function in noradrenergic and adrenergic cells [44]. Dopamine-deficient mice manifest normal dopamine neurons, neuronal connections [44] and D2 autoreceptors [63]. However, dopamine-deficient mice require daily injections of L-3,4-dihydroxyphenylalanine (L-dopa) for survival [44]. L-Dopa partially restores brain dopamine to $\sim$10% of normal in dopamine-deficient mice when measured 1 h after treatment but declines to $<1\%$ of control levels after 24 h [30, 44]. Without treatment, dopamine-deficient mice become severely hypophagic and die at $\sim$3 weeks of age. Systemic treatment with L-dopa rescues the mouse but produces a transient hyperactive state and induces robust immediate early gene expression in the striatum [64, 65], suggesting that dopamine deficiency results in hypersensitive D1 [64] and D2 receptors [30].

Mouse models of PD based on the expression of mutations known to cause the disease in humans offer a way to study the full extent of the PD pathology and to perform mechanistic studies by allowing the examination of early pathogenic steps in neurodegeneration [66]. Several rodent models for PD

have been created. Here we discuss models generated by manipulation of α-synuclein and parkin, two proteins involved in familial PD. A rare mutation in α-synuclein was the first genetic anomaly shown to cause familial PD [67]. This discovery led to the identification of α-synuclein, a vesicular protein that is a major component of Lewy bodies [68]. Mice over-expressing the normal or mutated forms of α-synuclein have been generated but their phenotype is highly variable, probably as a consequence of the different promoters used for the transgene [69]. Two mouse lines present with a neurochemical deficit in the nigrostriatal pathway and some behavioral anomalies [70, 71] provided compelling models to identify the role of α-synuclein over-expression [66]. In our laboratory we are examining alterations in corticostriatal synaptic activity in mice over-expressing α-synuclein under the Thy-1 promoter [70]. These mice exhibit a significantly lower frequency of spontaneous EPSCs compared with age-matched wild-type littermates [72]. In addition, whereas application of amphetamine reduces EPSC frequency in control mice, it has no effect in over-expressing mice suggesting that abnormal accumulation of α-synuclein alters glutamatergic activity in the corticostriatal pathway and its modulation by dopaminergic agents [72].

The second type of mutation shown to cause familial parkinsonism occurs in the gene encoding parkin, an E3 ligase [73]. Because parkin mutations are loss of function mutations, models have so far focused on parkin knockouts. In general, these mice show very mild anomalies [74, 75]. Mice defective in exon 3 show progressive motor anomalies when crossing a transverse beam as well as deficits in sensorimotor integration, starting as early as 2–4 months of age. Surprisingly, these mice have an increased basal release of dopamine in the striatum, and a reduced synaptic excitability in the striatum [74]. Overall, these defects are consistent with those observed in another line of mice with a similar mutation [75]. Neither mouse line, however, displays clear loss of dopaminergic or noradrenergic neurons. Therefore, similar to α-synuclein over-expressors, parkin knockout mice fall short of reproducing the full spectrum of anomalies observed in PD patients, in particular, the loss of nigrostriatal dopaminergic neurons.

Effects of Dopamine Deficiency on Postsynaptic Corticostriatal Activity

The effect of dopamine on the excitability of striatal cells is complex and depends on the interplay between dynamic intrinsic cellular and synaptic properties. MSSNs are relatively silent, fire spontaneously at a low rate and discharge in an episodic burst pattern largely in response to excitatory glutamatergic input. MSSNs demonstrate bistable behavior that acts as an input gating mechanism [76]. Membrane potentials alternate between a hyperpolarized down state (–85 mV), during which the neuron will not fire and a

depolarized active up-state (–60 mV) [77] where cortical information can be received and processed. This voltage-dependent bistable behavior is due to the interaction of the inward rectifying current and a phasic depolarizing input from the cortex (and thalamus) [77, 78]. When a large number of cortical neurons are activated simultaneously, large excitatory postsynaptic currents consisting mostly of AMPA/kainate and far fewer *N*-methyl-D-aspartate (NMDA) or metabotropic glutamate receptor (mGLUR) responses are induced. In response to this convergent excitatory input, MSSNs depolarize to the up state where additional excitatory input can generate an action potential [79]. State changes are maintained by D1 receptor stimulation, whereas D2 receptor activation reduces glutamatergic receptor responses [33]. The enhancing effects of D1 receptor activation appear to involve postsynaptic actions [32, 80, 81] whereas the attenuating effects mediated by D2 receptors involve both pre- and postsynaptic actions [29, 31–34]. This suggests that D1 receptors augment strong convergent excitatory synaptic inputs whereas D2 receptor activation suppresses the weak excitatory inputs [82, 83]. This mechanism reduces the signal-to-noise ratio for evoked activity and improves the spatial and temporal dynamics in long-term potentiation (LTP) and depression (LTD) [84–86].

The mechanisms by which dopamine produces differential effects on glutamatergic transmission are complex and involve modulation of voltage- and ligand-gated currents [87]. Our early studies demonstrated that the outcome of dopamine modulation depends not only on the type of dopamine receptor preferentially activated but also on the glutamate receptor subtype activated [32]. Thus, dopamine via D1 receptors enhances NMDA receptor-mediated responses whereas via D2 receptors it reduces AMPA receptor-mediated responses [34]. As D1 receptors predominate in MSSNs constituting the direct pathway, dopamine would promote motor programs by enhancing NMDA responses. In contrast, as D2 receptors predominate in MSSNs constituting the indirect pathway, it would inhibit motor output by reducing AMPA responses. This modulation is lost after dopamine depletion, as occurs in PD. Lack of dopamine will reduce the facilitatory effect on glutamatergic transmission in the direct pathway and will decrease the inhibitory effect in the indirect pathway. Disinhibition of indirect pathway neurons and reduced facilitation of direct pathway neurons could contribute to PD symptoms. Thus, alterations in dopamine availability may alter the balance of the direct and indirect pathways suppressing desired actions while promoting unwanted movements [7].

Effects of Dopamine Deficiency on Presynaptic Corticostriatal Activity

In addition to its effects on the MSSN, our laboratories and others have demonstrated that dopamine modulates corticostriatal neurotransmission presynaptically through D2 receptors [29–31, 33, 88, 89]. D2 receptors selectively

inhibit a subset of corticostriatal terminals with a low probability of release, while terminals with a high probability of release remain unperturbed [29, 30]. The effects of dopamine on corticostriatal release also increase with the frequency of cortical stimulation. Dopamine inhibition of glutamate release is minimal with low-frequency stimulation (1 Hz) but increases with higher rates of stimulation (20 Hz), with dopamine inhibition remaining selective for terminals with a lower probability of release. This dependence on frequency is also evident postsynaptically, with a reduction in evoked excitatory postsynaptic currents at higher rates of cortical stimulation [29]. Thus, dopamine acts as a low-pass filter, but the filtering is applied selectively to terminals with a low probability of release [83]. In this way, dopamine released by behaviorally salient stimuli can directly regulate striatal neurotransmission by selecting sets of corticostriatal projections and can process bursts of cortical information while rejecting others [29].

This striatal filtering by D2 receptors is altered in diseases such as PD that affect dopamine release. Initial data from dopamine-depleted rats and cats indicated that the firing rate of striatal neurons increased in the ipsilateral side of the lesion [90, 91]. Furthermore, after dopamine depletion spontaneous synaptic membrane depolarizations were observed [88, 89, 92], suggesting that presynaptic filtering by D2 receptors on corticostriatal terminals is obliterated in PD. Dopamine depletion also increases cell firing in striatopallidal neurons [93], decreases the threshold to evoke cortical responses [94] and facilitates the occurrence of cortically generated membrane oscillations in a subpopulation of striatal neurons [95]. It is thus likely that the facilitation in corticostriatal flow is selective to MSSNs of the indirect pathway. In fact, the cells of the direct pathway appear to have reduced spontaneous activity probably as a consequence of reduced cortical activity [93]. Abnormal corticostriatal filtering is also observed in D2 receptor-deficient mice [33]. In these mice, the frequency of spontaneous excitatory synaptic events is increased compared to control mice and large-amplitude membrane depolarizations are observed in a subset of neurons from D2 receptor-deficient mutants. In addition, morphological studies demonstrated that a subpopulation of MSSNs from D2 receptor-deficient mice displayed decreased dendritic spines compared with cells from control mice, providing evidence that D2 receptors play an important role in the regulation of glutamate receptor-mediated activity in the corticostriatal pathway and could function as gatekeepers of glutamate release, thus protecting striatal neurons from excessive excitation [33].

A recent report indicated that loss of spines in models of PD selectively affects D2 EGFP-positive MSSNs [55]. Although strong evidence was presented that this effect could be attributed to dysregulation of postsynaptic L-type Ca^{2+} channels, we believe that there is also a strong presynaptic contribution. As discussed above, DA-depleting lesions increase spontaneous glutamate-mediated synaptic activity. As this increase selectively affects D2 receptor-containing MSSNs, it is likely that excessive glutamate release becomes neurotoxic and induces spine elimination. Membrane loss can then induce increases in input resistance [88] making these cells even more excitable

and susceptible to glutamate release. Supporting experimental evidence for this idea was obtained recently by the demonstration that dendritic remodeling of MSSNs seen in models of PD occurs only secondary to increases in corticostriatal glutamatergic drive [96].

In dopamine-deficient states, the reduction in dopamine availability sensitizes presynaptic dopamine receptor responses [64, 92, 97] and likely influences cortical function by modifying cortical–basal ganglionic pathways. Optical recordings performed in our laboratory have determined the effect of dopamine deficiency and replenishment at single cortical synaptic terminals in mouse models for acute and chronic dopamine depletion [30]. Using reserpine-treated [30] and dopamine-deficient [44] mice, these investigations demonstrated that dopamine depletion produced sensitized presynaptic D2 receptor responses and altered dopamine-mediated responses from subsets of corticostriatal terminals [30]. In control mice, dopamine was found to inhibit ~85% of cortical terminals through D2 receptor actions that depressed exocytosis from terminals with a low probability of release. In contrast, for both reserpine-treated and dopamine-deficient mice, D2 receptor stimulation depressed release from both fast- and slow-releasing terminals. Since steady-state expression of the total population of D2 receptors is normal in dopamine-deficient mice [64], it is likely that the observable changes following dopamine deficiency reflect alterations in D2 receptor sensitization [43, 98]. Sensitized D2 receptor responses more broadly inhibited corticostriatal release and promoted further inhibition at the slow-releasing terminals. Similar alterations in dopamine receptor sensitivity [44] associated with dopamine deficiency in humans would likely lead to bradykinesia under dopamine-depleted conditions or dyskinesias following dopamine replenishment [30, 43].

The identification of functional D2 receptors on most corticostriatal terminals assists in understanding how the striatum encodes and relays information [83]. For instance, enhanced dopamine input during burst firing may prevent some striatal MSSNs from entering their depolarized 'up state' by depressing motor cortical input to the striatum. In dopamine-depleted states, dopamine receptor responses would become sensitized to repletion by L-dopa and disrupt normal striatal filtering. Such effects by presynaptic D2 receptors may underlie the untoward effects of L-dopa on voluntary movement and suggest how dopamine repletion in advanced stages of parkinsonism can disrupt cortical signaling due to normal behaviorally salient stimuli, leading to the development of dyskinesias.

Endocannabinoid-Mediated Modulation of the Corticostriatal Pathway

Evidence from experimental models of PD in rodents and primates indicates that there are profound changes in endocannabinoid receptor signaling in the basal ganglia circuits, both in the setting of dopamine depletion and

following L-dopa replacement therapy [99–104]. Endocannabinoid receptors are G-protein-coupled receptors expressed in high concentrations within the basal ganglia where they act as neuromodulators [105, 106]. Cannabinoid receptors are located on presynaptic corticostriatal projections [107] and on presynaptic terminals of GABAergic projections from MSSNs to the globus pallidus and substantia nigra pars reticulata [108].

In rodents, activation of dopamine receptors is accompanied by release of the endocannabinoid anandamide [109, 110], which may serve as an inhibitory feedback to counter dopamine-mediated behaviors [110, 111]. Endocannabinoids are released from target neurons in response to synaptic depolarization and act as retrograde signals that regulate synaptic transmission [112]. The modulatory functions of endocannabinoids are disrupted in parkinsonian patients [103] and following injury to the nigrostriatal pathway with 6-OHDA [102, 109] or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [103, 104], suggesting that subsequent alterations in endocannabinoid neurotransmission may also produce motor disturbances.

Endocannabinoids modulate corticostriatal synapses, indirectly at presynaptic corticostriatal terminals [113] and also directly at the MSSN [112]. Activation of postsynaptic D2 receptors in combination with metabotropic glutamate receptor stimulation inhibits presynaptic terminals through retrograde endocannabinoid signaling [113]. Postsynaptically, endocannabinoids released from neurons in the basal ganglia enhance GABAergic effects in animal models [114], presumably by reducing GABA reuptake [115]. Investigations using reserpine-exposed rats have demonstrated that dopamine depletion produces increased levels of endocannabinoids in the globus pallidus [116] with a compensatory reduction in endocannabinoid mRNA expression [117]. Stimulation of the endocannabinoid CB_1 receptors would be expected to reduce GABA reuptake and enhance GABAergic synaptic neurotransmission in the indirect pathway [117], contributing to the generation of parkinsonian symptoms [3, 118–120]. Moreover, D2 receptor activation on indirect pathway neurons is required for endocannabinoid-mediated LTD [54]. Indirect pathway eCB-LTD is absent in animal models of PD but is rescued by a D2 receptor agonist or inhibitors of endocannabinoid degradation [54]. Thus, reduced dopamine availability in PD and consequential changes in endocannabinoid activity would likely produce additional untoward signs and symptoms.

Acetylcholine-Mediated Modulation of Corticostriatal Afferents

One of the principal striatal targets of dopaminergic innervation are TANs that release acetylcholine and act to modulate both corticostriatal and dopaminergic activities. A longstanding maxim predicts that as striatal dopamine levels fall in parkinsonism, acetylcholine release rises [121]. Since acetylcholine efflux from TANs appears to regulate glutamate from most corticostriatal terminals by

stimulating α7*-nicotinic receptors [15, 19], alterations in dopamine availability would likely affect corticostriatal activity. TANs are the main source of acetylcholine in the striatum but they represent only 1–2% of the total neuronal population [122]. They are important neuronal integrators, critical for plasticity [17], reward-mediated associative learning [123] and drug-seeking behaviors [86, 124]. TANs are characterized by their slow spontaneous activity and long-duration action potentials that are triggered by small (1–5 mV) depolarizing potentials [125].

TANs possess inhibitory D2 receptors [126–128] and are also modulated by excitatory D1 receptors [126, 129, 130]. Dopamine interactions on TAN activity mediates corticostriatal responses [131] including dopamine-dependent corticostriatal LTD [132] via presynaptic receptors found on corticostriatal terminals, including M2-type muscarinic acetylcholine receptors (mAChRs) that are inhibitory [16–18] and α7*-type nicotinic acetylcholine receptors (nAChRs) that exert tonic excitation [15, 19]. Acetylcholine also potentiates dopamine release through lower affinity β2*-nicotinic receptors on nigrostriatal terminals [133] and regulates its own release via M4 autoreceptors [16, 134].

Until recently, the prevailing view was that a progressive decrease in striatal dopamine would increase acetylcholine efflux secondary to the lack of inhibitory tone on TANs [135, 136]. D2 receptors on TANs reduce cholinergic efflux by diminishing the opening of Cav2 Ca^{2+} channels in response to membrane depolarization [126]. Thus, a lack of D2 receptor stimulation would not likely drive acetylcholine levels upward. Instead, a reduction in dopamine availability increases striatal acetylcholine concentrations by attenuation of M4 muscarinic autoreceptor coupling to Cav2 Ca^{2+} channels that regulate acetylcholine release and spiking [137].

As nAChRs are rapidly desensitized at high agonist levels, an increase in acetylcholine availability would desensitize lower affinity β2* nAChRs and possibly higher affinity receptors including α7*-nAChR. Since nAChRs can regulate or activate other G-protein receptors including dopamine receptors [133] and mAChR [19], these synaptic changes would produce further alterations in both corticostriatal and dopaminergic activities.

Summary

By regulating corticostriatal activity, dopamine allows complex filtering of cortical information entering the basal ganglia. Corticostriatal synapses are presynaptically inhibited by dopamine, an effect mediated by D2 receptors [29]. The most active cortical terminals are selectively resistant to dopamine inhibition, while the majority of terminals are inhibited. Since the effects of dopamine on corticostriatal release increase with the frequency of cortical stimulation, dopamine acts as a low-pass filter, but the filtering is applied selectively to terminals with a low probability of release. This suggests

dopamine release associated with salience during motor learning modifies motor commands by reinforcing specific corticostriatal connections and filtering out less effective cortical inputs [83]. Postsynaptically, dopamine D1 receptor activation promotes motor programs by enhancing NMDA responses whereas D2 receptor activation inhibits motor output by reducing AMPA responses. In PD, a reduction in dopamine availability promotes dopamine receptor hypersensitivity with impairment of presynaptic filtering and postsynaptic signal processing.

The absence of dopamine-mediated corticostriatal filtering in dopamine-depleted states may underlie the inability of episodic dopamine repletion to completely correct movement disabilities as rapid alterations in striatal dopamine availability lead to dysregulation of cortical and striatal activities [138]. Furthermore, the production of sensitized dopamine receptor responses may explain how chronic treatment with L-dopa might lead to long-term synaptic plasticity and to the development of dyskinesias [30]. Under dopamine-depleted conditions, dopamine receptor hypersensitivity also induces dopamine receptor-mediated inhibition of terminals with a high probability of release. Thus, minute increases in synaptic dopamine would cause large decrements in excitatory cortical transmission. As such, pharmacological treatment with L-dopa for treatment of parkinsonism would induce striatal depression. This phenomenon may be best appreciated in L-dopa-responsive dystonia where relatively small maintenance doses of L-dopa are required to reduce bradykinesis and dystonia while preventing severe motor dyskinesias otherwise observed in doses typical for treatment of PD. Similarly, the maintenance of stable brain dopamine concentrations in PD may reduce untoward pharmacological responses including dyskinesias often seen following increases of L-dopa [64].

Dopamine also modulates corticostriatal activity indirectly through other intrinsic striatal neurotransmitters including acetylcholine [137, 139], adenosine [140], endocannabinoids [54] and serotonin [141]. Although alterations in these neurotransmitters likely provide additional motor dysregulation, they may also provide an alternative or additional option for therapeutic intervention [54, 139, 140].

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Chapter 7
Cholinergic Interneuron and Parkinsonism

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Abstract Recent advances in our knowledge of striatal function revealed a previously unexpected role for striatal cholinergic interneurons. The recognition that interneurons are essential in synaptic plasticity and motor learning represents a significant progress in deciphering how the striatum processes cortical inputs, and why pathological circumstances cause motor dysfunction. Loss of the reciprocal modulation between dopaminergic inputs and the intrinsic cholinergic innervation within the striatum represents a suitable explanation for the efficacy of anticholinergic drugs both in Parkinson's disease and in dystonia. These advances provide exciting indications to the underlying circuit alterations. In this chapter, we discuss the experimental and clinical evidence in attempt to clarify how alterations in striatal cholinergic signalling may contribute to motor dysfunction and ultimately to identify novel therapeutic strategies to fine-tune cholinergic signalling in basal ganglia disorders.

Introduction

The basal ganglia represent key structures involved in motor, cognitive, memory and motivational processes [1]. These brain areas consist of distinct loops proceeding along parallel pathways linking cortical and subcortical regions sub-serving sensorimotor, associative and affective tasks. Disorders involving basal ganglia regions show a clinical heterogeneity, with a predominant motor impairment. Irrespective of the pathogenic background, that includes dysfunction in mitochondrial energy metabolism, glutamate excitotoxicity, oxidative damage and genetic determinants, the functional rearrangements in basal ganglia circuitry occurring in course of movement disorders led to the traditional classification into *hyper*- and *hypo*-kinetic movement disorders [2–6]. Based on

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experimental and clinical evidence, this classification predicted the existence of an imbalance within basal ganglia loops which is believed to account for the *hyper*- and *hypo*-kinetic clinical manifestations in patients. In each of these disorders, such rearrangements were accompanied by profound biochemical modifications, which have represented peculiar hallmarks of the disease, such as dopamine depletion in Parkinson's disease (PD). Knowledge of these biochemical changes has significantly facilitated the development of animal models, able to reproduce motor dysfunction both in non-human primates and in rodents.

In the striatum, the main input stage of basal ganglia, neuronal subtypes are classically divided into projection neurons, medium spiny neurons (MS), the majority of striatal cells (95%) and a subset of interneurons. Four types of interneurons have been described: three of them are GABAergic and the other one is cholinergic, which account for only 2% of all striatal neurons [7]. Striatal acetylcholine (ACh) has been shown to exert a central role in motor control and synaptic plasticity [8]. Experimental evidence showed that integrity of nigrostriatal dopaminergic pathway is essential for cholinergic activity. In fact, in normal conditions, cholinergic and dopaminergic neurotransmission are in balance in the striatum, with a tonic dopaminergic inhibitory tone on ACh release [9]. Dysfunction of the nigrostriatal pathway, as it occurs in PD, has been shown to induce important changes in cholinergic transmission at striatal level. Accordingly, anticholinergic drugs have been utilized in clinical practice in different pathological conditions such as PD and dystonia [10, 11]. Thus, a renewed interest for striatal cholinergic system emerged in the recent past, and both experimental and clinical evidence are in support of this assumption [8].

In this chapter we will discuss the role of cholinergic interneurons in the pathogenesis of PD and related disorders.

Cholinergic Interneurons: From Morphological Clues to Functional Evidence

The largest neuronal cells in the striatum were identified as *giant interneurons* by Kolliker in his classic studies of Golgi stained material since the late 1800s [12]. They are now known to be cholinergic neuron on the basis of choline acetyltransferase immunolabelling [7] and represent only a small fraction of the total neuronal cell in the neostriatum ($\sim$2%). These cells have a large soma ($\sim$40 μm) and extremely dense axonal processes when compared to other striatal cells. The dense and widespread local axon collaterals are largely restricted to the neostriatal matrix where they primarily target the MS neurons. Thus, they belong to the subclass of striatal cells known as interneurons, together with parvalbumin interneuron (also known as fast-spiking cells, FS), neuropeptide Y, nitric oxide synthase and somatostatin interneurons and calretinin interneurons [7]. The widespread dendritic and axonal fields indicate that cholinergic neurons can integrate synaptic inputs over relatively large regions

and act either as modulators of the excitability of projection neurons or as associative interneurons. The advent of the in vitro brain slice preparation, combined with optical techniques, has allowed not only the characterization of the intrinsic properties of these cells but also a detailed investigation of the pharmacological actions of several neurotransmitters on cholinergic interneurons. In vitro electrophysiological recordings have shown that cholinergic interneurons are autonomously active, firing in the absence of synaptic input, and can exhibit a typical rhythmic pause firing pattern in which action potential spikes are terminated periodically by large hyperpolarization (AHP) [13], and show a prominent sag in response to hyperpolarizing-current injection owing to activation of hyperpolarization-activated cationic current (I_h). Because striatal cholinergic interneurons are local interneurons, their axons do not innervate basal ganglia output structure; therefore, it is presumed that the tonic firing and the pauses of cholinergic interneurons influence striatal circuitry by modifying the activity of MS projection neurons.

Pioneering in vivo recordings have shown that two types of neurons may be distinguished in the monkey striatum according to modulation of their firing to the presentation of stimuli of behavioural significance. The first type is represented by the majority of striatal neurons, the MS projection neurons, whereas the second type has been defined as tonically active neurons (TANs), which show a regular tonic firing of 3–9 Hz in the absence of movements, a mean interspike interval of about 150–200 ms and a tendency to discharge synchronously in pairs [14–16]. Mapping of TANs in the striosome-matrix compartmentation of the striatum has shown that they are preferentially concentrated in the matrix and at the striosome-matrix border. The density and spatial positions of TANs recorded along with single penetrations of a recording electrode in the striatum of behaving monkeys have led some authors to suggest that among acetylcholine-, somatostatine-, parvalbumin- and carlretinin-positive interneurons, the cholinergic interneurons are the most likely neurons corresponding to TANs [17]. Notably, TANs appear to respond directly to primary reward and their responses are strongly influenced by the behavioural context in which the animals are required to perform. More recently, it has been shown that independently of the motivational context, other factors can influence the activity of TANs. In particular, TANs might have a broader role, being able to select the appropriate motor behaviour related to environmental events [18].

Animal Models of Parkinson's Disease

PD is a common neurodegenerative disease with a complex etiology which results from a combination of both genetic and environmental factors [3, 4]. Selective degeneration of dopamine neurons in the substantia nigra (SN) is responsible for the appearance of motor symptoms. In order to understand the underlying mechanisms and to develop new therapies for PD, it is important to

have available animal models that recapitulate both motor symptoms as well as the slow progression of the disease as accurately as possible. Although these models have proven useful, none of them reproduce all the features of the human disease. Such limitation has generated criticisms on the reliability of the models. However, an animal model does not necessarily have to precisely parallel its human counterpart. On the contrary, a model should help clarifying single pathogenic steps of the disease, and ultimately identify cellular and molecular targets for testing novel therapeutic agents.

MPTP Model

Systemically administered 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) crosses the blood–brain barrier and is converted by monoamine oxidase B (MAO-B) to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP). MPDP then spontaneously oxidizes to a toxic metabolite, 1-methyl-4-phenylpyridinium (MPP^+). Once released into the extracellular space, MPP^+ can be taken up by the dopamine transporter into the dopaminergic neurons. MPP^+ then accumulates into mitochondria where it inhibits complex I of the electron transport chain leading to impairment of cell respiration and to neuronal death [19]. MPTP is used both in non-human primates and in mice. Chronic treatment of monkeys with low doses of MPTP causes a gradual neuronal loss in the SN, a pattern better suited to modelling human PD. Indeed, systemic administration of MPTP induces a bilateral parkinsonian syndrome in monkeys, with clinical manifestations reproducing several features of human PD, including tremor, rigidity, bradykinesia, and postural instability. Additionally, the response to L-DOPA treatment and the development of motor complications during long-term therapy are also similar to those seen in PD patients. However, in spite of the consistent dopaminergic neuronal loss observed in PD, the peculiar Lewy bodies have not been reported to occur in humans or monkeys acutely intoxicated by MPTP [20]. Recently, a mouse model of chronic PD was proposed, consisting of a continuous MPTP infusion via osmotic pumps. MPTP infusion led to progressive behavioural changes, inhibition of the ubiquitin-proteasome system and generation of neuronal inclusions containing ubiquitin and α-synuclein [21].

Rotenone Model

The selective complex I inhibitor, rotenone, is extremely hydrophobic and can cross the cell membrane and enter the cytoplasmic space, independently of dopamine transporter activity. Thus, rotenone seems well suited to induce a widespread complex I inhibition in experimental animal models [22]. Accordingly, chronic systemic infusion of rotenone induces a syndrome characterized

by rigidity and akinesia. Furthermore nigral neurons have been reported to show Lewy body-like inclusions, containing α-synuclein and ubiquitin [22, 23]. However, there is not a general agreement on the rotenone model of PD [24, 25]. Some authors claim that rotenone reproduces a pattern of diffuse degeneration, showing no specificity for the nigrostriatal pathway. Further work is required to better define the potentiality of this model.

6-Hydroxydopamine Model

6-Hydroxydopamine (6-OHDA) was the first animal model of PD developed to reproduce motor dysfunction in rodents [26]. Because 6-OHDA does not cross the blood–brain barrier, it is administered into the SN *pars compacta*, the median forebrain bundle, or the striatum. In various animal species, 6-OHDA-induced toxicity is relatively selective for monoaminergic neurons, as a result of its uptake by dopamine and noradrenaline transporters [27]. At cellular level, 6-OHDA produces toxic effects by inhibiting mitochondrial complex I and causing oxidative stress [28, 29]. The extent of the lesion can be quantified by measuring the asymmetric turning behaviour induced by systemic L-DOPA administration [30, 31]. The 6-OHDA model has proven useful as it provided a considerable amount of information at biochemical, morphological and functional level. Dopamine denervation is known to affect corticostriatal glutamatergic neurotransmission [32, 33]. In vitro studies from 6-OHDA lesioned rats showed that striatal projection neurons undergo severe functional impairment, as they loose both forms of long-term synaptic plasticity, potentiation (LTP) and depression (LTD) [34]. In the parkinsonian rat, the symptomatic L-DOPA therapy is able to restore the alterations observed in glutamatergic neurotransmission [34, 35]. As in humans, however, after prolonged L-DOPA treatment dyskinesia appears. The cellular mechanism underlying these involuntary movements has been proposed to reside in the loss of bidirectional synaptic plasticity by striatal projection neurons [36]. L-DOPA treatment restored both LTD and LTP, but failed to rescue corticostriatal synaptic depotentiation in dyskinetic rats.

Cholinergic Interneurons and Parkinsonism

Anticholinergic drugs represented the first accepted pharmacological treatment for PD [11]. The use of antimuscarinic agents was justified by the observation of a functional antagonism between striatal dopamine and ACh [37]. Dopamine regulates striatal cholinergic tone through activation of dopamine D2, which results in a reduction in ACh release [9]. Loss of the dopaminergic innervation causes an imbalance between dopamine and ACh in the striatum, with an ensuing increase in cholinergic signalling. In 6-OHDA-denervated rats,

Mackenzie et al. observed a clear increase of striatal ACh and a decrease of D2 receptor expression [38]. A functional impairment has also been reported in non-human primates. In MPTP-treated monkeys, depletion in dopamine content drastically reduces the expression of acquired responses to reward stimuli suggesting a dysfunction in TANs activity [16, 17]. The restore of TANs responses by the dopaminergic agonist apomorphine further supports that dopamine is essential in shaping TANs responses to reward-related events. In spite of such evidence, the cellular mechanisms underlying this functional rearrangement are not entirely understood. In a recent work by Ding and coworkers [39], dopamine depletion does not influence D2 receptor signalling, but reduces M4 muscarinic autoreceptor function, leading to an enhancement of neuronal activity as result of ACh release [39]. At the molecular level, RGS-4, a GTP-ase–accelerating protein seems to have a pivotal role in the regulation of M4 autoreceptor signal transduction. Ding and colleagues showed that dopamine depletion causes an increase of intracellular RGS-4 protein expresion which, in turn, induces a reduction in M4 autoreceptor activity [39]. Loss of autoreceptor function could result in an increased ACh release that, by acting on ACh receptors expressed on sorrounding MS, would lead to an abnormal striatal output and ultimately to the emergence of motor symptoms.

Role of Cholinergic Interneurons in Other Basal Ganglia Disorders

Progressive supranuclear palsy (PSP) is a neurodegenerative disorder characterized by parkinsonism, subcortical dementia and supranuclear gaze palsy [40]. Convincing evidence supports a generalized impairment of mitochondrial function as a major pathogenic determinant of this disorder [41]. Moreover, it has been suggested that a disruption of cholinergic transmission could account for clinical symptoms observed in PSP patients [42]. Accordingly, the levels of the striatal vesicular acetylcholine transporters, a reliable marker of cholinergic activity, are severely reduced in striatal samples from PSP patients, a finding consistent with the loss of striatal cholinergic interneurons [43]. In addition, previous studies have shown a decrease of striatal choline acetyltransferase (ChAT), suggesting that a loss of cholinergic neurons is a neuropathological hallmark of PSP [44].

The effects of mitochondrial inhibition have been recently investigated in cholinergic interneurons in a striatal slice preparation [45]. Application of the mitochondrial toxin rotenone induced an early membrane hyperpolarization coupled to a fall in input resistance and a rise in intracellular sodium levels, which was followed by a later rise in intracellular calcium. The pharmacological analysis suggested that rotenone, by disrupting the ATP content, leads to a decreased Na^+-K^+ATPase function and, in turn, to intracellular sodium overload. ATP-sensitive and sodium-activated potassium channels caused the

following membrane hyperpolarization. Cell count from rotenone-treated slices confirmed a large decrease in the number of ChAT immunoreactive cells. These data are in support of a selective involvement of the cholinergic systems, in particular the striatal cholinergic transmission.

Dystonia is a neurological syndrome characterized by repetitive muscle contractions and twisting movements and frequently complicated with abnormal postures [46]. Often it represents a complication of other disorders such as PD. Besides PD, anticholinergic drugs have proven effective also in dystonia, with patients showing a moderate benefit in both focal and generalized forms of dystonia [10, 47]. Although currently available muscarinic antagonists are not devoid of serious side effects, their therapeutic relevance warrants further research aimed at obtaining more selective muscarinic drugs.

It is believed that functional abnormalities at striatal level may be responsible for the induction of dystonia. Notably, in mice overexpressing mutant torsin-A, the mutation found in DYT1 dystonia, we found an important alteration in the response of cholinergic interneurons to D2 dopamine receptor activation [48]. Normally, D2 receptor signalling reduces interneuronal firing (see above). Conversely, D2 receptor activation increased cholinergic interneurons activity in mice overexpressing mutant torsin-A [48]. This difference could be attributed to an enhanced inhibitory coupling of D2 receptors to Cav2 calcium channels that regulate the opening of Ca^{2+}-dependent potassium channels [48]. The D2 receptor-dependent increase in interneuronal activity could lead to increase striatal ACh release, providing a rationale for the efficacy of anticholinergic drugs in the treatment of dystonia.

Huntington's disease (HD) is an autosomal dominant disease, characterized by choreiform movements, severe behavioural and cognitive impairment. It is caused by an abnormal expansion of CAG trinucleotide repeats in the gene coding for huntingtin [49]. Traditionally, it was shown that cholinergic interneurons are selectively spared in this disease. However, it has been recently demonstrated that in the striatum of HD transgenic mice, the levels of VAChT and ChAT are significantly reduced [50]. A marked reduction of cholinergic markers was found also post-mortem in the striatum of HD patients [50, 51]. These results further strengthen the hypothesis of a major involvement of cholinergic interneurons in disorders affecting the basal ganglia.

Future Perspectives

Experimental and clinical evidence emerged in favour of a critical involvement of the striatum in many physiological and pathological conditions. In animal models of human diseases involving the basal ganglia, such as PD and dystonia, cholinergic interneurons seems to play a major role in the adaptive mechanisms occurring in the striatum. Several experimental findings converge to indicate that an adequate endogenous ACh tone is central to striatal function and that

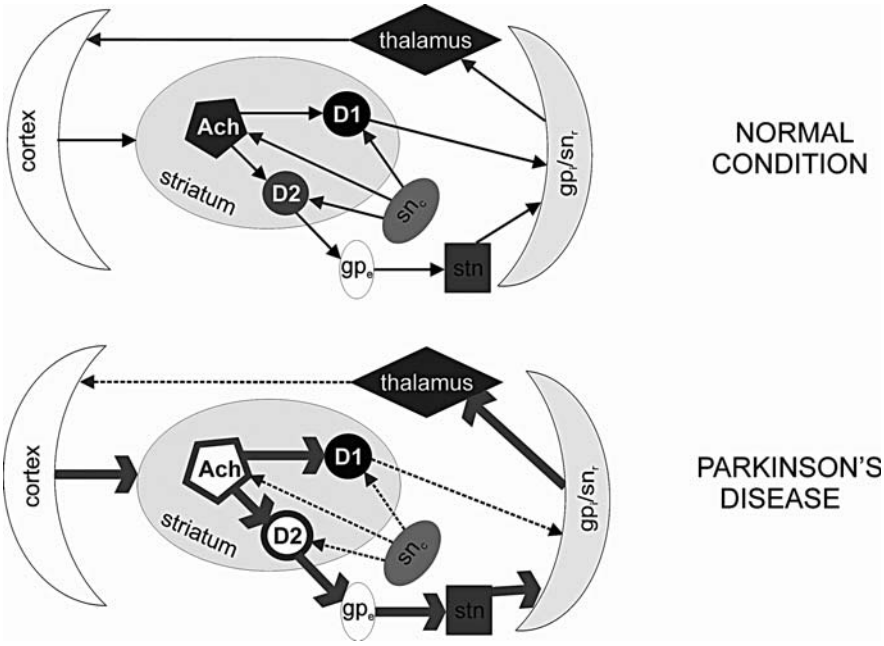

Fig. 7.1 Simplified representation of basal ganglia in physiological condition and in parkinsonism. (**A)** In normal conditions, both MS projection neurons and cholinergic interneurons receive glutamatergic and dopaminergic inputs, from cortex and substantia nigra pars compacta (SNc), respectively. Both D1- and D2-containing MS neurons are contacted by cholinergic interneurons. D1-containing MS neurons that give rise to the direct pathway project to the internal segment of the globus pallidus (gpi) and to substantia nigra pars reticulata (SNr), whereas the indirect pathway establishes synaptic connections between the D2-containing MS projection neurons and both the external portion of the gp (gpe) and the subthalamic nucleus (stn). In the parkinsonian state, the loss of dopaminergic inputs leads to an enhanced cholinergic signalling that in turn produces disinhibition of D2-receptor-containing MS neurons. Both the glutamatergic inputs from the cortex to the striatum and from the STN to the output nuclei are significantly enhanced. The ensuing imbalance of the indirect pathway leads to an increased inhibitory output to the thalamus, and to a decreased thalamocortical feedback. These rearrangements result in the hypo-kinetic motor symptoms of parkinsonism

changes in such regulation could result in pathological rearrangements in the whole basal ganglia circuitry (Fig. 7.1). Therapeutic strategies targeting cholinergic signalling in movement disorders, such as PD and dystonia, should be taken into account in order to restore a balanced striatal network activity. However, to date, pharmacological tools developed disappoint expectations. According to recent experimental work, muscarinic M4 autoreceptors seem to be an interesting therapeutic target for PD. In fact, a dysregulation in M4 autoreceptor function has been recently demonstrated to underlie the alteration in cholinergic signalling observed in response to dopamine depletion. Similarly, in dystonia, the abnormal cholinergic transmission observed in mice with the DYT1 mutation could benefit from an enhanced muscarinic autoreceptor function.

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Chapter 8
Basal Ganglia Network Synchronization in Animal Models of Parkinson's Disease

Judith R. Walters and Debra A. Bergstrom

Introduction

The critical role of dopamine cell loss in the etiology of Parkinson's disease has been well documented. However, understanding how loss of dopamine ultimately disrupts activity in cortico-subcortical networks and induces motor dysfunction remains a challenge. The present chapter reviews studies in a rodent model of Parkinson's disease investigating effects of dopamine receptor stimulation on firing rate, pattern and synchronization of activity in cortico-subcortical networks involving the basal ganglia. In particular, these studies focus on how tonic increases and decreases in dopamine receptor stimulation alter passage of slow oscillatory activity through basal ganglia circuits as a step toward elucidating the role of dopamine in modulating subcortical network dynamics.

The highest concentration of dopamine in the brain is in the basal ganglia. The dopamine neurons that degenerate in Parkinson's disease are located in the pars compacta of the substantia nigra (SNpc) and terminate mainly in the striatum, where they synapse most prominently on spines of medium spiny neurons, in close proximity to the glutamatergic terminals [1, 2] of corticostriatal and thalamostriatal projections. Modest dopaminergic projections to the subthalamic nucleus (STN), the internal segment of the globus pallidus (GPi) (analogous to the rodent entopeduncular nucleus [EPN]) and the external segment of the globus pallidus (GPe) provide the possibility of direct dopaminergic regulation of other basal ganglia areas [3–8].

An important goal in Parkinson's disease research has been to determine how dopamine neurons, through their robust projections to the striatum and more modest projections to other basal ganglia nuclei, modulate basal ganglia output, and thereby affect the function of downstream motor circuits. Clearly,

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some tonic level of dopamine receptor stimulation is critical to the maintenance of normal motor activity [9, 10]. Phasic release of dopamine is critically involved in basic mechanisms of attention and learning in association with novel and salient events [11–15], but the role of dopamine in facilitating familiar movements appears more dependent on tonic levels of dopamine receptor stimulation than on precisely timed bursts. A major task in understanding Parkinson's disease, therefore, is delineating how appropriate rates of dopamine receptor stimulation maintain normal motor function and how too much or too little promotes dysfunction.

Dopamine Effects on Rate in Basal Ganglia Circuits

Data from receptor-binding studies, in situ hybridization techniques and advances in dopamine receptor cloning were integrated in the late 1980s–early 1990s to provide an operating model of basal ganglia function [16–18]. This early model, referred to as the "dual circuit", "rate" or "Albin DeLong" model, became widely used in generating hypotheses regarding the effects of changes in striatal dopamine receptor stimulation on basal ganglia output (Fig. 8.1). Increased stimulation of the D2 dopamine receptor subtype, preferentially expressed by striatal projections to the GPe, was predicted to attenuate activity

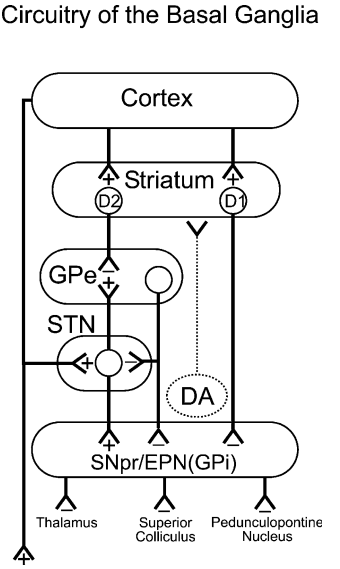

Fig. 8.1 Schematic diagram of the circuitry of basal ganglia. Abbreviations: GPe, globus pallidus external; STN, subthalamic nucleus; DA, dopamine neurons of the substantia nigra pars compacta; SNpr, substantia nigra pars reticulata; GPi, globus pallidus internal; EPN, entopeduncular nucleus; "+" excitatory transmission; "–" inhibitory transmission

in striatopallidal projections and induce a net decrease in basal ganglia output through modulation of the indirect pathway. Increased stimulation of the D1 receptor subtype, preferentially expressed by the striatal projections to the substantia nigra pars reticulata (SNpr) and GPi, was predicted to similarly induce a net decrease in basal ganglia output through potentiating activity in the direct pathway [16, 19, 20].

A therapeutically relevant prediction of the rate model was that the dysfunctional effects of dopamine agonist administration and dopamine cell loss on movement correlate with decreases and increases, respectively, in firing rates of neurons in basal ganglia output nuclei, the GPi and the SNpr. Decreases in inhibitory output from the basal ganglia and disinhibition of thalamocortical activity were linked to hyperactivity, while increases in inhibitory output from the basal ganglia and inhibition of thalamocortical activity were linked to paucity of movement in Parkinson's disease. The idea that basal ganglia output neurons were overactive following loss of dopamine provided the rationale for lesioning the GPi as a treatment for advanced Parkinson's disease [21–24]. The success of this approach led investigators to examine the efficacy of lesioning the STN, directly upstream from the GPi. Subsequently, deep brain stimulation (DBS) was applied to these nuclei as a reversible strategy for altering basal ganglia output [21, 22, 24–31]. In parallel with the clinical success of these procedures, data obtained from on-going studies in animal models of Parkinson's disease and eventually from Parkinson's disease patients during implantation of electrodes for lesion or DBS treatment allowed further evaluation of the rate-based model's predictions.

Testing Predictions of the Rate-Based Model: Effects of Dopamine Agonists

Drugs selective for D1 and D2 receptor subtypes became available in the mid-1980s, making it possible to examine the predictions of the rate-based dual circuit model. Neurophysiological recording studies conducted in an immobilized, locally anesthetized rat preparation showed that systemically administered dopamine agonists or dopamine uptake blockers triggered robust increases in mean firing rate in the GPe, as the rate model predicted. However, this increase in GPe firing rate only occurred with non-selective D1/D2 agonists or when both D1 and D2 selective agonists were co-administered [32–39]. In fact, D2 agonists alone exerted only modest rate increasing effects on GPe firing rates, and these modest effects appeared dependent on intact endogenous dopamine tone at D1 receptors [37]. Similarly, given alone, a D1 agonist also induced modest rate increasing effects on GPe activity. And, surprisingly, D1 agonists enhanced activity of STN neurons, while D2 agonists had little effect [40–43].

These neurophysiological results were notable for being inconsistent with the rate model's predictions that D1 agonists would have little effect on firing rates

of GPe and STN neurons in the indirect pathway, while D2 agonists would increase tonic activity in the GPe and reduce tonic activity in the STN. Other results from these studies were also at odds with predictions of the rate model. For example, given alone, D1 agonists induced only mild increases in activity in the SNpr, while drugs stimulating both D1 and D2 receptors exerted a mix of increases, decreases and no change [39, 44–46].

On the other hand, the effects of D1 and D2 agonists on mean firing rates in the GPe were consistent with observations emerging from behavioral studies showing that concurrent stimulation of D1 and D2 receptor subtypes was required to induce the behavioral hyperactivity associated with dopamine agonist treatment [36, 47–49]. Taken together, the behavioral and neurophysiological studies highlighted the importance of synergistic interactions between D1 and D2 receptor subtypes in mediating functionally significant effects of dopamine, but left unclear how and where these interactions take place.

Testing Predictions of the Rate-Based Model: Effects of Dopamine Loss

The effects of dopamine cell lesion on mean firing rates of basal ganglia nuclei were also explored in the locally anesthetized, immobilized rat. In rats with unilateral dopamine cell lesions, two results stood out as strikingly consistent with the predictions of the rate model and different from observations in the intact rat. First, in the absence of any drug treatment, mean firing rates in the STN were dramatically increased following dopamine cell lesion [41–43, 50]. This observation was in agreement with results from primate models of Parkinson's disease [25, 51–53], where, as described above, increases in STN firing rates provided a strong rationale for exploring the therapeutic potential of pallidotomy and subthalamotomy in Parkinson's disease, and the subsequent application of DBS in these structures.

Second, in contrast to the variable rate changes observed in intact rats, substantial decreases in SNpr firing rates were observed after administration of the combination of D1 and D2 agonists in dopamine-lesioned rats [39, 46, 54]. This enhanced response to D1/D2 agonist treatment after dopamine cell lesion was consistent with the idea that loss of dopamine-induced compensatory increases in response to dopamine receptor stimulation in the striatum, producing enhanced inhibitory input to the SNpr via the striatonigral inhibitory pathway.

While baseline increases in STN activity and agonist-mediated decreases in SNpr activity in lesioned rats were consistent with the rate model predictions, there were also observations in the dopamine-lesioned rat that were inconsistent. For example, dopamine lesion-induced increases in STN firing rates were puzzlingly unaccompanied by significant changes in firing rates of the SNpr and EPN [46, 55], and D1 agonists alone still induced increases in STN firing rates; stimulation of both D1 and D2 receptors appeared required to reduce firing

rates in the overactive STN after dopamine cell lesion [41]. Thus, while the overall effects of altering tonic dopamine receptor stimulation on basal ganglia output were more consistent with predictions of the rate-based model in dopamine cell-lesioned rats than in intact rats [39, 44–46, 54, 56–58], these results did not support the idea that differential rate changes in the direct and indirect pathways and simple increases or decreases in basal ganglia output could account for the hyperactivity and hypoactivity induced by tonic changes in dopamine receptor activation. At the circuit level, rate-based models were limited in explaining how loss of dopamine promotes both bradykinesia and tremor, how D1 and D2 receptor-mediated effects interact to induce stereotypy, how intermittent dopamine receptor stimulation promotes dyskinesias and how lesions of the GPi alleviate not only the hypokinetic aspects of Parkinson's disease but also the hyperkinetic symptoms [22, 29, 59–61].

Dopamine Effects on Firing Pattern in Basal Ganglia Circuits

The rate-based perspective emerged as a pioneering but limited framework for explaining how dopamine loss affects basal ganglia output, and data from a variety of sources further highlighted the complexity of basal ganglia circuitry. Attention was directed to the STN as an input nucleus to the basal ganglia [62–65], and data supported co-localization as well as segregation of D1/D2 receptor subtypes in the striatum [19, 20, 66, 67], additional dopamine receptor subtypes [68], and distributed connections between basal ganglia nuclei [69, 70]. Meanwhile, neurophysiological investigations, aided by software that allowed online assessment of neuronal activity in time and frequency domains, explored dopamine's ability to modulate cortico-subcortical dynamics in the context of firing pattern and synchronization of neuronal activity throughout the basal ganglia network.

Multisecond Oscillations

Examination of firing rate changes in basal ganglia neurons over relatively long-time periods, seconds and minutes as opposed to milliseconds, demonstrated that increased stimulation of dopamine receptors dramatically affects synchronization of activity in basal ganglia circuits in the ultraslow frequency range. In the locally anesthetized, immobilized rat preparation, 30–70% of spike trains recorded in the SNpr, GPe, EPN and STN and 20% in the SNpc show oscillatory activity with very slow periodicity, in the range of 2–60 s (0.017–0.5 Hz) [50, 71–77]. While it is still not clear what processes generate these ultraslow oscillations, it has been suggested that activity in this frequency range may prime intracellular mechanisms to promote plasticity [78, 79]. More

recently, fMRI imaging techniques have used ultraslow oscillations in brain function to identify brain networks that show correlated activity during "resting states" and task performance [80–85]. More relevant to the subject of the present volume, these multisecond oscillations in basal ganglia spike trains provided insight into the impact of dopamine receptor stimulation on subcortical circuit dynamics.

Dopamine Agonist Effects on Incidence and Frequency of Multisecond Oscillations

While dopamine cell lesion did not significantly alter the properties of the multisecond oscillations in spike trains from the SNpr, STN, EPN or GPe [75, 76, 86, 87], increases in dopamine receptor stimulation robustly and selectively affected the frequency, spectral power and incidence of these oscillations in basal ganglia activity in the immobilized rodent preparation. Systemic administration of the D1/D2 agonist apomorphine, the selective dopamine uptake blocker GBR-12909 or less selective uptake blockers such as methylphenidate (Ritalin), cocaine or d-amphetamine induced oscillations with shorter periods, typically 10–15 s as opposed to pre-drug values of ~30 s, and increased the power (amplitude and regularity) of the oscillations [50, 71–76, 87, 88].

These dopamimetic drug effects were relatively selective for dopamine, as desipramine, an uptake blocker selective for norepinephrine, and fluoxetine, an uptake blocker selective for serotonin, were not effective at changing the properties of ultraslow oscillations [73]. The effects of dopamine agonists were also centrally mediated, as the peripheral D2 antagonist domperidone had minimal ability to reverse the effects of dopamine agonists on the amplitude or frequency of these firing rate oscillations [71]. Moreover, in rats with unilateral dopamine cell lesions, dopamine agonists induced changes in multisecond oscillations in the lesioned hemisphere at doses substantially lower than those required for intact rats [75, 76], further indicating that the agonists were acting centrally, at the sites where dopamine receptors were denervated, to modulate the multisecond oscillations. Intriguingly, in these unilaterally lesioned rats, low doses of dopamine agonists were also effective in altering the parameters of multisecond oscillations in EPN spike trains in the contralateral, non-lesioned hemisphere (Ruskin et al., unpublished observations), showing that changes in oscillatory activity triggered by supersensitive dopamine receptors in one hemisphere could influence basal ganglia function in the other hemisphere.

The effects of dopamine agonists on multisecond oscillations in the basal ganglia also demonstrated D1/D2 receptor synergism, as the greatest changes in incidence and frequency occurred after combined treatment with doses of selective D1 and D2 agonists which individually were relatively ineffective [72]. These results paralleled the D1/D2 receptor synergism observed with respect to GPe firing rate and hyperactivity and stereotypy in rodents [36] and argued that the effects of dopamine on ultraslow oscillatory firing patterns are relevant to dopamine's effects on basal ganglia output and motor activity.

Dopamine Agonist Effects on Synchronization of Multisecond Oscillations in GPe, STN and SNpr

The studies described above suggested that correlations between multisecond oscillations within and between interconnected nuclei could provide insight into the effects of dopamine receptor stimulation on the dynamics of the basal ganglia network in this frequency range. Data from simultaneous recordings of pairs of neurons in the STN and GPe nuclei in opposite hemispheres, and in GPe-STN and GPe-SNpr nuclei in the same hemisphere showed that firing rate oscillations of individual neurons are frequently (~30%) correlated in the 2–60 s timescale and provided evidence that neurons participating in these oscillations are widespread throughout the basal ganglia [74, 77, 89, 90]. When correlated oscillations were observed, phase relationships, viewed in the context of STN output being excitatory and GPe output being inhibitory, indicated that in some cases the GPe was shaping oscillatory activity in the STN, and in other cases the reverse was observed. Similarly, the data did not identify either of these nuclei as having the dominant role in shaping the multisecond oscillatory activity observed in SNpr spike trains in baseline conditions.

Dopamine agonist administration, however, had dramatic effects on the transmission and properties of multisecond oscillations in basal ganglia circuits. Paired recordings supported a role for dopamine in modulating phase relationships of oscillatory activity in the basal ganglia network (Fig. 8.2). Marked increases were observed in the incidence of neuronal pairs showing correlated oscillations, oscillations in STN and GPe spike trains became more frequently in phase and oscillations in the GPe and SNpr became more synchronized (~100% of pairs) and more consistently antiphasic after dopamine agonist administration [74, 89].

These results suggest that increasing the level of dopamine receptor stimulation affects the relationship between the GPe and the STN in the ultraslow frequency range, with the STN more consistently determining the phase of the oscillations in the GPe. Under these conditions the data support a model of oscillation flow through the basal ganglia circuit in which oscillations in the GPe are shaped by STN input and have the potential of entraining oscillatory activity in the SNpr, especially if the oscillations from the GPe arrive in phase with oscillations via the direct pathway from the striatum. In support of this hypothesis, lesion of the STN nucleus disrupted the consistent phase relationship between the GPe and the SNpr induced by dopamine agonist administration [74].

Evidence for ultraslow oscillations in basal ganglia spike trains provides support for the physiological significance of ultraslow oscillations in fMRI imaging studies and the use of these fluctuations to assess correlated activity in neuronal circuits [80–85]. These observations also raise questions for future research on the role for ultraslow oscillations in organizing faster frequencies in the basal ganglia network [77]. However, while these results address the impact

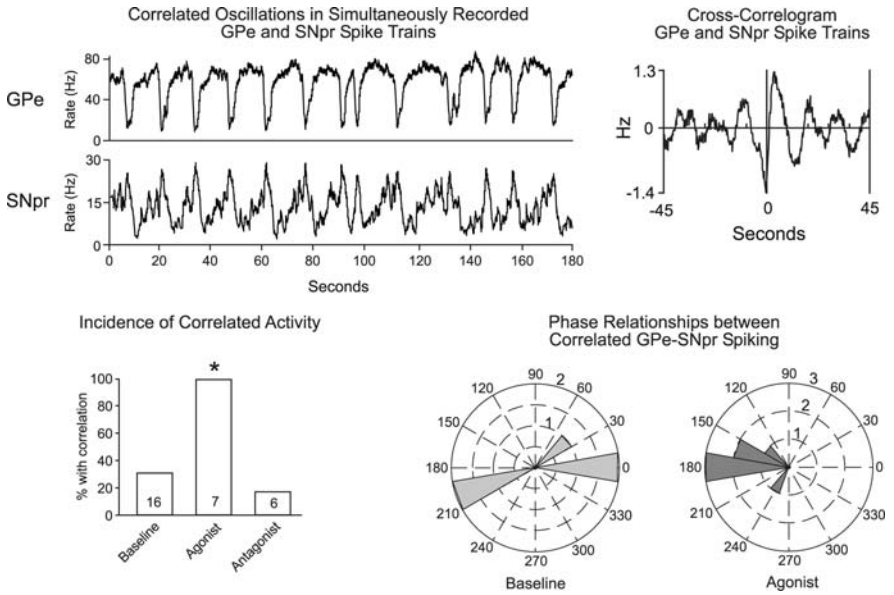

Fig. 8.2 Correlated multisecond oscillations between GPe and SNpr firing rates in intact rats. Under baseline conditions, multisecond oscillations in firing rate of simultaneously recorded GPe and SNpr spike trains were significantly correlated in 5 of 16 recorded pairs in baseline. Of the five correlated pairs, phase relationships were bimodal, either ~0° or 180°, as depicted in the polar histogram. Following D1/D2 dopamine agonist (apomorphine) administration, multisecond oscillations were correlated in 100% of the pairs (a significant increase from baseline) and became antiphasic, as depicted in the polar histogram. An example of correlated GPe/SNpr spike trains following apomorphine administration (*upper left*) with their GPe-triggered SNpr cross-correlation (*upper right*) is presented. *Bar graph* shows that dopamine antagonist (haloperidol or eticlopride) administration reversed the agonist-induced increase in correlated multisecond incidence. *Significantly different from baseline and antagonist treatment. Data from Ruskin et al. [58, 74]

of increased dopamine receptor stimulation on the dynamics of basal ganglia networks, they do not provide insight into how loss of dopamine affects activity in the frequency domain in the basal ganglia circuits.

1 Hz Oscillations

Further perspective on effects of dopamine loss on basal ganglia activity has emerged from a collection of studies focusing on oscillatory activity in the 1 Hz range. Cortical activity in systemically anesthetized animals, as reflected by cortical EEG and local field potential (LFP) recordings, is highly synchronized and characterized by slow ~1 Hz oscillations [91–96]. As this synchronized cortical state is relatively stable over time, it can be viewed as a probe or input

signal for assessing effects of dopamine loss on the passage of oscillatory activity through basal ganglia networks.

Dopamine Cell Lesion Effects on 1 Hz Oscillations in the Basal Ganglia

A number of studies have reported that firing patterns in the striatum, GPe, STN, EPN and SNpr become more bursty in anesthetized rats with unilateral 6-hydroxydopamine (6-OHDA)-induced dopamine cell lesion (Figs. 8.3 and 8.4) [97–122]. This bursty activity has been shown to be correlated with slow oscillations in cortical EEG/LFP [106, 107, 112, 114, 120–123].

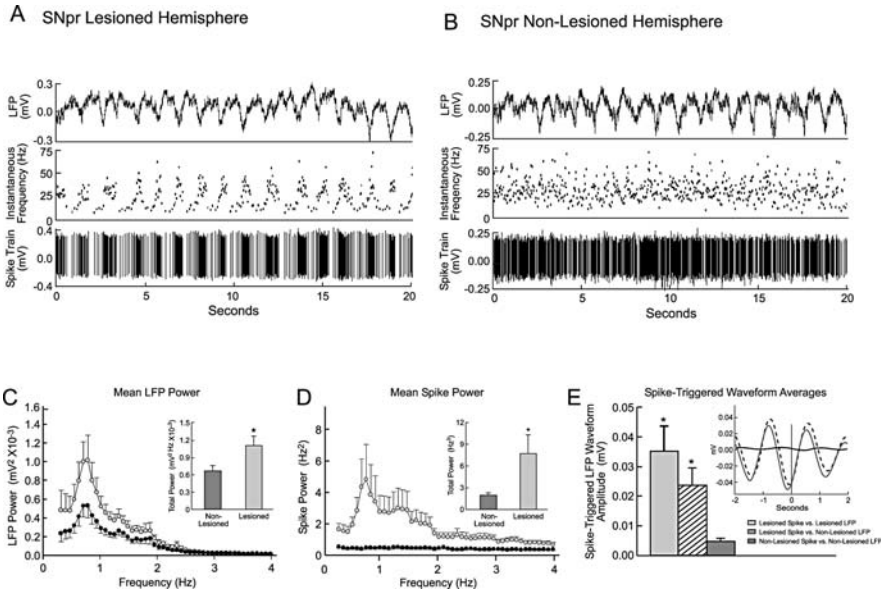

Fig. 8.3 Simultaneous recordings of SNpr spike trains and SNpr LFPs ipsilateral (**A**) and contralateral (**B**) to a unilateral dopamine cell lesion in an urethane-anesthetized rat. SNpr spike trains recorded in the lesioned hemisphere exhibited marked burstiness; spectral analysis showed that these firing patterns were significantly more oscillatory than those in the non-lesioned hemisphere (**D**). Peak frequencies in the slow ~1 Hz range were recorded in the SNpr LFPs in both non-lesioned and lesioned hemispheres (**C**); these slow oscillations were similar to those observed in SNpr spike trains from the lesioned hemisphere. SNpr spike-triggered waveform averages illustrated that SNpr spikes occurred most frequently at or near the trough of the SNpr LFP in the lesioned hemisphere (from recording in **A**, shown in **E** inset) and showed significantly greater correlations between SNpr spike train firing pattern and LFP oscillations (**E**) in the lesioned hemisphere than those in the non-lesioned hemisphere. Correlations between SNpr spike train activity from the lesioned hemisphere and SNpr LFP oscillations from the non-lesioned hemisphere were also significantly greater than spike train/LFP correlations from the non-lesioned hemisphere. *Significantly different from non-lesioned

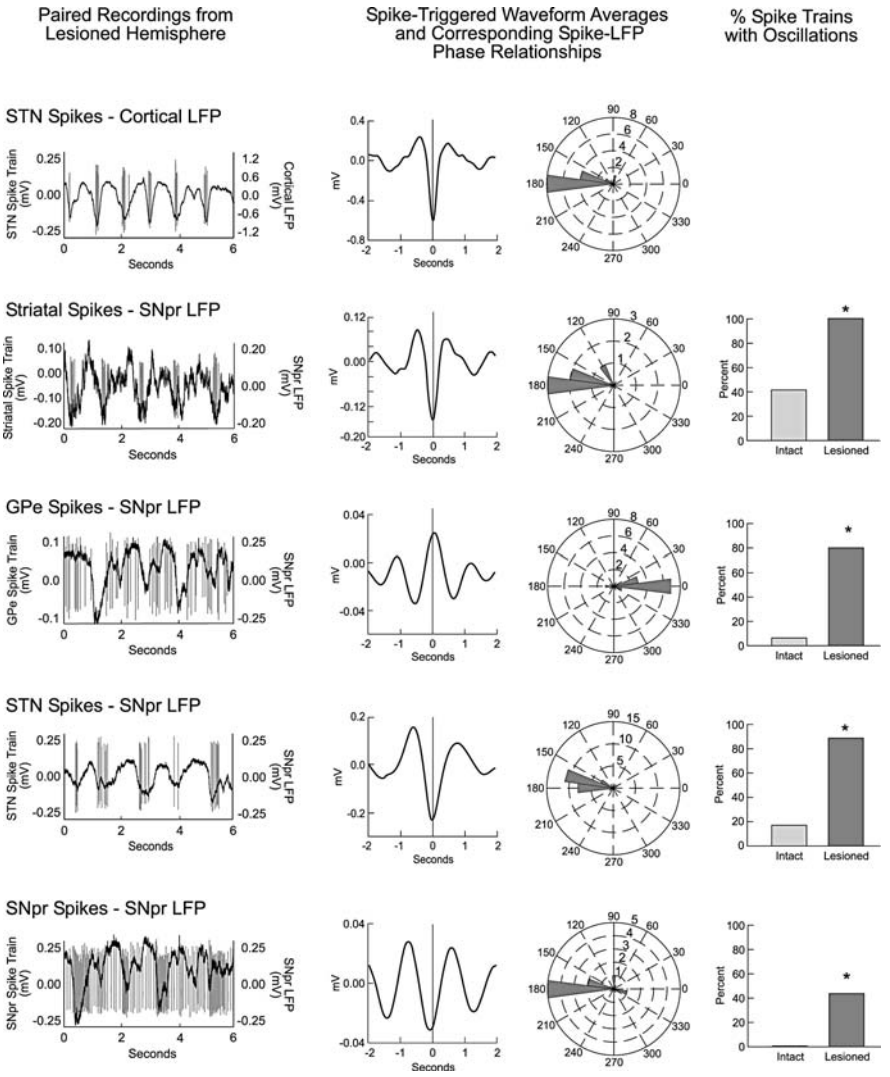

Fig. 8.4 Paired basal ganglia spike train and cortical LFP or SNpr LFP recordings in unilateral dopamine cell-lesioned rats anesthetized with urethane. Simultaneous recordings of STN spike trains with cortical LFPs (*top left*) demonstrated that STN spikes occurred most frequently at or near the trough (~180°) of the cortical LFP oscillation, as also illustrated by the STN spike-triggered cortical LFP waveform average (*middle*). Polar histogram (*right*) summarizes the distribution of phases of STN spikes with respect to cortical LFP for all significantly oscillating STN spike trains recorded with cortical LFPs. Similar recordings and data are presented for SNpr LFPs with striatal, GPe, STN or SNpr spike trains. In contrast to the other recordings, simultaneous recordings of GPe spike with SNpr LFPs showed that GPe spiking coincided with the peak (0°) of the SNpr LFP oscillations as also illustrated by the GPe spike-triggered SNpr LFP waveform averages. The number of striatal, GPe, STN and SNpr spike trains with significant oscillations in the 0.3–2.5 Hz range was significantly greater in lesioned rats than intact rats (*bar graphs on right*). Data are based on 300 s epochs; spike train/LFP examples are representative 6 s epochs of 300 s epochs. *Significantly different from intact. Data from Walters et al. [121]

Murer, Tseng and coworkers provided important insight into this phenomenon by demonstrating that striatal neurons are more depolarized after dopamine cell lesion and fire more frequently in conjunction with the slow oscillations in cortical EEG in anesthetized rats [106, 112]. These observations, together with additional contributions of Mallet and coworkers [123] argue that after loss of dopamine, alteration in striatal processing of oscillatory cortical input is an important factor in the emergence of the 1 Hz synchronized and oscillatory activity in the basal ganglia network of anesthetized rats.

Dopamine Cell Lesion Effects on Phase Relationships of 1 Hz Oscillations in Basal Ganglia Circuits

To further examine how loss of dopamine affects passage of oscillatory activity through the excitatory and inhibitory pathways within the basal ganglia network, spike trains recorded from the striatum, GPe, STN and SNpr were paired with simultaneous LFP recordings from the SNpr or motor cortex ipsilateral to a unilateral lesion of substantia nigra dopamine neurons in urethane-anesthetized rats (Fig. 8.4). LFPs in the SNpr and cortex were used as references to establish phase relationships between alterations in oscillatory activity in the other nuclei [121].

Analyses of phase relationships between slow oscillations in striatal, GPe, STN and SNpr spike trains, with SNpr LFP as a common reference, showed that dopamine loss results in (a) increased oscillatory activity in most GPe spike trains consistent with increased phasic inhibitory input from the striatum, (b) increased oscillatory activity in STN spike trains consistent with convergent oscillatory input from cortex and GPe and (c) increased oscillatory activity in $\sim$50% the SNpr spike trains consistent with convergent inhibitory and excitatory oscillatory input from the GPe and STN, respectively. These results argue, therefore, that the oscillatory firing patterns that emerge in the basal ganglia output nuclei in anesthetized rats after dopamine cell lesion are shaped by several factors: the temporal characteristics of cortical activity, changes in striatal processing of cortical input, decreased transmission of cortical activity through the direct pathway and increased transmission through the indirect pathway, convergence of cortical input to the STN via the hyperdirect pathway with opposite phase input from the GPe, and finally, convergence of input from the STN with opposite phase input from the GPe at downstream sites in the SNpr and EPN [121] (Figs. 8.4 and 8.5).

Other processes may also contribute to the emergence of oscillatory and bursty activity in basal ganglia output in the anesthetized animal model of Parkinson's disease. These include direct effects of dopaminergic denervation on local processes within the STN [124] and indirect effects of dopamine loss on basal ganglia output affecting activity and coherence in thalamocortical projections and cortical networks that may contribute to changes in basal ganglia activity [123, 125, 126]. Results to date do not support a substantial role for increases in oscillatory activity in cortical or thalamic input to the basal ganglia in mediating the increases in 1 Hz oscillatory activity in the basal ganglia

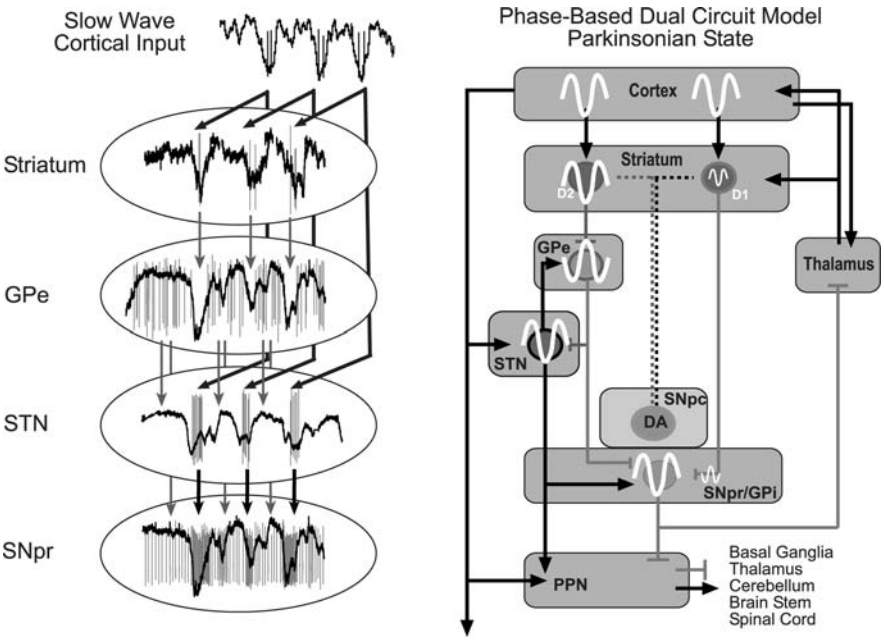

Fig. 8.5 Phase model illustratinga hypothesized scheme for passage of oscillatory signals in the slow 0.3–2.5 Hz range through the cortico-basal ganglia pathway after dopamine loss in anesthetized rats. *Black lines* indicate excitatory connections between nuclei and *gray lines* indicate inhibitory connections. Examples of striatal, GP, STN and SNpr spiking activity recorded simultaneously with SNpr LFP are shown with SNpr LFP as a common temporal reference (indicated in *black*). Cortical oscillatory inputs to the striatum and STN are indicated. Results are consistent with loss of dopamine enhancing transmission of oscillatory activity primarily through the indirect pathway, resulting in robust oscillatory activity in the SNpr. Implied in this model are (1) D2 receptor-bearing striatopallidal neurons transmitting patterned activity from the cortex to the GP, (2) striatally mediated pauses in inhibitory GP activity contributing to the timing of bursts in STN neuronal activity, and (3) pauses in inhibitory GP output coinciding with bursts in excitatory STN output supporting enhanced oscillatory activity in SNpr/GPi spike trains

network in the anesthetized, unilaterally lesioned rodent model of Parkinson's disease [119, 127]. However, data from Mallet and coworkers suggest that indirect effects of dopamine loss may contribute to imbalance in basal ganglia networks by reducing cortical input to the direct striatonigral pathway [123].

Determining the impact of changes in basal ganglia output on downstream activity is important to further understanding of processes leading to motor dysfunction in Parkinson's disease and the unilaterally lesioned rat model of Parkinson's disease may be useful for providing some insight into this question [119, 127]. In this context, it is interesting that alterations in basal ganglia output in the anesthetized rat with unilateral dopamine cell lesion have a significant effect on activity in an area which has become a new target for DBS in the treatment of Parkinson's disease, the pedunculopontine nucleus

[128] (Fig. 8.6). Changes in spike timing with respect to slow wave oscillations in the motor cortex are observed after dopamine cell lesion, consistent with a modulatory effect of increased oscillatory input from the SNpr.

The hypothesis that loss of dopamine facilitates passage of synchronized activity through the basal ganglia network by disrupting striatal "filtering" of oscillatory components of cortical input [112] provides a useful framework for envisioning how loss of dopamine alters basal ganglia dynamics. This model incorporates aspects of the older rate model, predicting that loss of dopamine should reduce activity in the direct pathway and facilitate activity in the indirect pathway, and adds input from the hyperdirect pathway and consideration of phase relationships to the overall dynamics (Fig. 8.5). However, it is well recognized that slow 1 Hz oscillations do not dominate cortical activity in

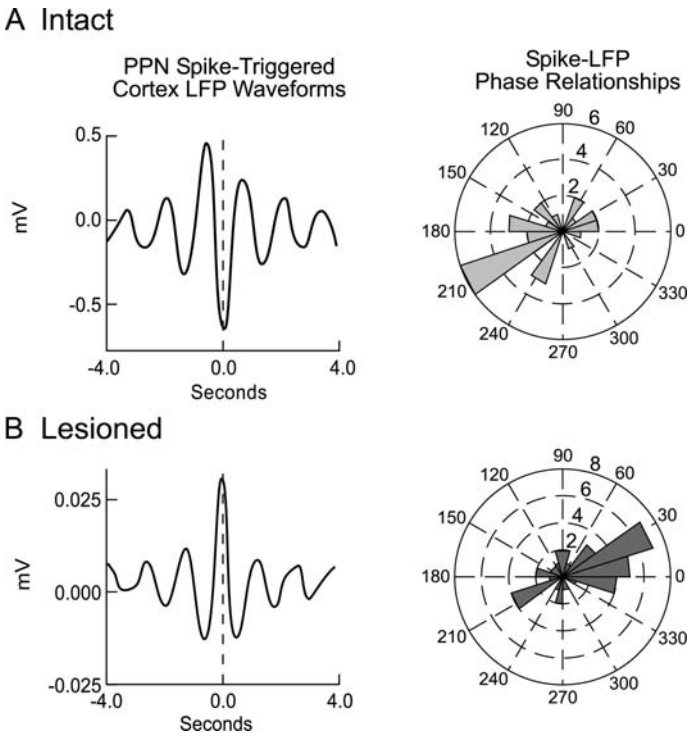

Fig. 8.6 PPN spike timing relative to cortical LFP oscillatory activity in urethane-anesthetized rats. PPN spike trains were recorded simultaneously with LFPs from the motor cortex, layer V in intact rats and unilateral dopamine cell-lesioned rats. PPN spike-triggered LFP waveform averages illustrate the time of PPN spiking relative to the phase of LFP oscillatory activity. PPN spiking occurred at or near the trough (~180°) of motor cortex LFP activity in intact rats, as summarized in the polar histogram. Following dopamine cell lesion, this phase relationship changed significantly as PPN spiking occurred primarily at the peaks (~0°) of LFP oscillations. *Significant unimodal distributions of phase relationships between PPN spiking and LFP activity. Data from Aravamuthan et al. [128]

awake parkinsonian animals or Parkinson's disease patients. This model's predictive validity with respect to the emergence of faster frequency oscillations in the basal ganglia network in Parkinson's disease remains to be determined.

4–30 Hz Oscillations

The studies described above demonstrate that changes in tonic levels of dopamine receptor stimulation have significant effects on the dynamics of basal ganglia network activity in slow and ultraslow frequency ranges. Research in animal models of Parkinson's disease and recordings in parkinsonian patients show that dopamine loss is also associated with pronounced effects on faster frequency activity in basal ganglia circuits. Understanding the role of dopamine loss in the emergence and dysfunctional effects of oscillations in the 4–30 Hz range is a current challenge in on-going efforts to understand factors influencing cortico-subcortical dynamics in Parkinson's disease.

Dopamine Cell Lesion Effects on 4–30 Hz Oscillations

In the late 1970s, Filion [129] interrupted the nigrostriatal dopamine pathway in monkeys with electrolytic lesion and described changes in firing patterns in the globus pallidus. Subsequently, the discovery that MPTP selectively destroys dopamine neurons in the monkey [130–133] led to valuable experiments examining effects of dopamine loss on firing rate and pattern in the basal ganglia of the non-human primate. In addition to increases in rate which were most notable in the STN, MPTP studies reported increased synchronization in neuronal spiking and oscillatory firing patterns in the 4–18 Hz frequency ranges in the GPi, GPe and STN after dopamine loss [51, 52, 134, 135–142].

A growing body of data collected from the GPi and STN in parkinsonian patients during implantation of DBS electrodes has also supported the view that, in addition to changes in firing rate, abnormal firing patterns are associated with loss of dopamine in Parkinson's disease. These firing patterns include increased bursting and abnormal oscillatory activity that is related to limb tremor [143–146]. Oscillations in the beta frequency range (12–30 Hz) are of interest in the context of akinesia, as power in this range in STN LFPs in parkinsonian patients during rest is reduced by L-dopa treatment and movement [147–163].

Similar changes in firing pattern have also been observed in the awake, locally anesthetized immobilized rat model of Parkinson's disease. In this model, loss of dopamine has been associated with increased incidence of oscillations in neuronal firing rates in the 4–18 Hz range in the EPN nucleus [55]. In addition, spike/LFP relationships reflect significant increases in correlated spiking activity within and between the GPe and the STN in the beta frequency range (15–30 Hz) after dopamine cell lesion [90](Fig. 8.7). Measures of

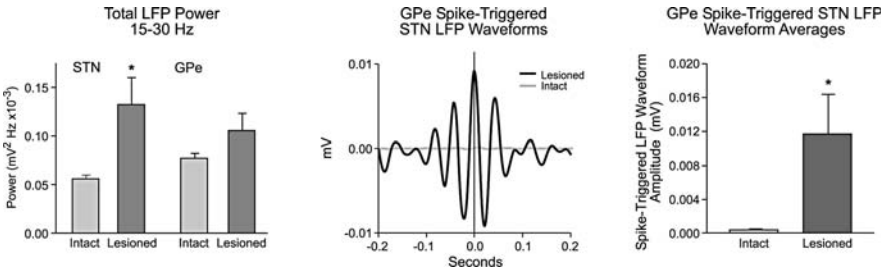

Fig. 8.7 Data from simultaneous recordings in the STN and GPe in intact rats and rats with unilateral dopamine cell lesions. A significant increase in power was observed throughout the beta frequency range (15–30 Hz) in STN LFPs of lesioned rats compared with intact rats. GPe LFP power was significantly increased in the 15–18 Hz range in lesioned rats compared with intact rats. GPe spiking and STN beta range LFPs show increased correlation after loss of dopamine; amplitude of GPe spike-triggered beta range STN LFP waveforms from lesioned rats were significantly greater than those from intact rats (n = 7–9 pairs). *Significantly different from intact. Data from Tierney et al. [90]

coherence in STN and GPe in simultaneous single-unit recordings from these two nuclei support the hypothesis that relationships between STN and GPe are altered by dopamine cell lesion with GPe having a greater influence on temporal dynamics of STN activity after dopamine loss [90]. As reflected in spike-triggered waveform averages, GPe spiking becomes more synchronized with STN LFP oscillations in the beta frequency range after dopamine cell lesion. Power in this frequency range in STN spiking is also increased, as is coherence between LFPs in GPe and STN in lesioned animals. These results also show that dopamine agonist administration has a desynchronizing effect on spike/LFP synchronization in the beta frequency range in the GPe and STN nuclei in both intact and lesioned rats, which is reversed by dopamine antagonist administration.

These observations have been extended to the awake behaving rat in studies recording spiking and LFP activity from chronic indwelling electrodes in the STN [164–167] and more recently bilaterally in SNpr nuclei in rats with unilateral dopamine cell lesion [168, 169]. In the latter study, rats were trained to walk on a circular treadmill and data are collected during alternating rest and walk periods. Significantly increased beta frequency activity was observed during rest in the SNpr of the lesioned hemisphere, relative to walking periods and relative to rest in the non-lesioned hemisphere and in control rats. Spiking activity in the SNpr was more correlated with the beta activity in the lesioned hemisphere, indicating that the increased synchronized and oscillatory activity observed in the STN [165, 167] is transmitted to the SNpr and affects basal ganglia output. Interestingly, a higher frequency peak (25–40 Hz) was selectively observed in the lesioned hemisphere in the SNpr during walking, suggesting that dysfunctional oscillatory activity in the high beta/low gamma range might also be expressed in this nucleus during effort to walk in the dopamine-lesioned rat [169]. These

observations support the view that rodent models of Parkinson's disease have potential for probing the significance and source of faster frequency oscillations in the basal ganglia in Parkinson's disease.

Conclusion

Data reviewed above show that dopamine receptor-driven processes regulate transmission and synchronization of ultraslow, slow and faster frequency oscillatory activity in basal ganglia circuits. Altering levels of dopamine receptor stimulation, through removal of dopaminergic input or administration of dopamine agonists, affects not only firing rates in the basal ganglia but also dynamics of synchronized and oscillatory activity within this network and in basal ganglia output. However, the relationship between processes generating these oscillations and dopamine's ability to modulate their transmission through basal ganglia circuits is not well understood. This question has been well explored with regard to the 1 Hz slow waves associated with anesthesia. These oscillations emerge in the context of the anesthetized state, presumably through effects of anesthetics on cortical network dynamics [96]. The increased transmission of the 1 Hz oscillations through the basal ganglia after dopamine cell death appears related to changes in striatal processing of cortical input as a result of dopaminergic denervation of striatal neurons [109, 112, 121, 123]. Phase relationships of oscillatory activity recorded at different points in the basal ganglia circuit argue that the indirect pathway provides increased transmission of the oscillatory activity, while transmission through the direct pathway is diminished. This is consistent with a reduced impact of dopamine on D2 receptors mediating inhibitory effects on transmission through the striatopallidal projection and on D1 receptors mediating excitatory effects on transmission through the direct striatonigral projection.

The phase-based dual circuit model discussed above (Fig. 8.5) is effective in accounting for the transmission of the slow wave 1 Hz oscillations, but its relevance to the emergence of faster frequency oscillatory activity in basal ganglia nuclei after dopamine loss remains to be determined. Studies in brain slices support the view that loss of dopaminergic innervation in the STN may contribute to changes in STN that promote STN/GPe resonance in the beta frequency range [170, 171]. Moreover, data from recordings in the GPe in rats with dopamine cell lesions in ketamine-/urethane-anesthetized rats [107] suggest that collateral circuitry in the GPe provides the temporal dynamics required to sustain GPe–STN oscillations. In addition, a role of striatal processing in transmission of faster frequency oscillations has emerged recently from a study by Dejean and colleagues [172] who have made effective use of the 5–13 Hz oscillations in high-voltage spindles recorded in the basal ganglia and cortex of awake chronically implanted rats to further probe the effect of dopamine loss on basal ganglia dynamics. Phase relationships imply increased

transmission of the 5–13 Hz oscillations through the indirect pathway and a greater impact of input from the hyperdirect pathway on activity in the SNpr after dopamine loss, similar to processes suggested for transmission of the 1 Hz oscillations. This study also reports increased coherence between cortex and striatum in the beta frequency range suggesting a role for the striatum in transmission of beta activity in the basal ganglia after loss of dopamine.

In summary, data support a critical role for dopamine in modulating organization of synchronized and oscillatory activity in basal ganglia networks. However, many questions remain [163]. These include how synchronized and oscillatory activity emerges in the basal ganglia, whether this activity results primarily from alterations in striatal filtering of cortical input or is generated by local changes in dopamine input engendering reverberations in subcomponents of the basal ganglia network. Finally, critical and unresolved questions include how alterations in basal ganglia output affect downstream circuits and the significance of these changes with respect to symptoms of Parkinson's disease.

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Chapter 9
Converging into a Unified Model of Parkinson's Disease Pathophysiology

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Early models of basal ganglia functional organization pointed at changes in spontaneous activity as the underlying basis of akinesia, the main clinical manifestation of Parkinson's disease. The "classical" model posits that an imbalance between the direct and indirect pathways results in an increase in the average firing rate of basal ganglia output neurons, tonic inhibition of motor thalamo-cortical circuits, and reduced motor output [1, 2]. However, after nearly 20 years most researchers in the field would probably agree in that there is little evidence to support this hypothesis [3, 4]. Current models posit that dopamine depletion impedes movement by promoting excessive oscillatory synchronization of basal ganglia neurons. According to this view, spontaneous oscillation and synchronization could induce resonance at certain frequencies, precluding the encoding of other frequencies more relevant to movement [5] and/or spatial segregation of information flow [6]. Nevertheless, the mechanism underlying abnormal oscillatory synchronization is a matter of debate. Current models point at intrinsic oscillations in the GP-STN network [7, 8] or at changes in the gain of pathways conveying and reinforcing cortical oscillations [9, 10].

An alternative model puts forward the idea that basal ganglia neurons would not encode correctly action instructions in parkinsonian patients when they try to move [11]. Dopamine depletion would alter the fine temporal and spatial coordination of information flow through trans-striatal and trans-subthalamic pathways, resulting in an increased inhibitory influence on the thalamus. Evidence to support this model is scarce, since studying movement in Parkinson's disease is very difficult. Also, views about the mechanisms involved vary, with focus on either the trans-striatal or trans-subthalamic pathways [11, 12].

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Recent studies performed in the rat 6-hydroxydopamine (6-OHDA) unilateral lesion model provided valuable information regarding what changes take place in basal ganglia activity after dopamine depletion and what mechanisms underlie them. These studies are based on very simple grounds. First, dopamine-depleted rats exhibit behavioral deficits resembling those observed in patients [13]. Second, anesthesia allows the emergence of spontaneous network dynamics resembling those seen in physiological conditions while minimizing confounds inherent to behavioral states (uncontrolled internal states, environmental influences, unnoticed movements, proprioceptive feedback). Third, electrical stimulation of the motor cortex allows studying the coordination of information flow through trans-basal ganglia pathways in response to phasic and focal incoming signals. In conjunction with some recent computational studies and experiments performed in behaving monkeys and rats, this work helps to understand how functional alterations of basal ganglia activity arise and which is their contribution to the clinical manifestations of Parkinson's disease.

Slow Waves Versus Activation or Silent Versus Depolarized Network States?

Under urethane anesthesia, cortical spontaneous activity alternates between two main states, slow wave activity and an activated or desynchronized state. These cortical states show striking similarities to those seen during natural slow wave sleep and alertness respectively [14]. During slow waves, either natural or induced by urethane, cortical neurons alternate synchronously between a silent hyperpolarized DOWN state and a depolarized UP state that enables the firing of action potentials. On the other hand, cortical activation, as seen in non-anesthetized restrained animals or under urethane, is related to a persistently depolarized state that allows a more or less tonic firing in pyramidal neurons.

Striatal medium spiny neurons (MSNs) also show alternation between UP and DOWN states in vivo. It has long been accepted that cortical afferents induce the UP state in the striatum, since MSNs do not show UP states in brain slices or after severing cortical inputs [15, 16]. However, the temporal and spatial relationships between activity in the cerebral cortex and striatum, which were postulated to be precisely organized [17, 18], have not been elucidated until recently. It is now clear that, in MSNs, the archetypical rhythmic alternation between UP and DOWN states correlates with slow wave activity in the cerebral cortex [19, 20], and that it is replaced by a persistently depolarized state during cortical activation [21] see also [22]. What is more, cortical input not only dictates the onset of UP states but is necessary for sustaining them. Indeed, if the cortex stops firing spontaneously or is turned off artificially, MSNs fall into the DOWN state.

Remarkably, the sub-threshold behavior of MSNs recorded under urethane resembles very closely that seen in non-anesthetized restrained rats [22]. More exhaustive studies by Kasanetz et al. [23] revealed a very tight temporal coupling between activity in cortical ensembles and the UP states of MSNs (Fig. 9.1).

A straightforward reading of these studies could be that, when we analyze the activity of basal ganglia neurons under slow waves induced by anesthetics, we will get information that is relevant only to slow wave sleep. In this context, Kasanetz et al. studies [21, 23] indicate that slow wave activity spreads from the thalamo-cortical system into the striatum, where each MSN indexes the functional state of afferent cortical ensembles in the form of UP states. More recent studies indicate that this spreading is spatially organized following gradients rather than strictly segregated channels [24]. In the perspective of current views about segregation of information processing during behavior, this could mean that information is processed within a different associational context in slow wave sleep.

However, there is another way of looking at the slow waves, much like as two figure patterns can be seen in the famous paintings by Escher, frogs or fishes, depending on what is taken for background. By dissecting the slow wave, the behavior of basal ganglia neurons can be studied separately during epochs of high (UP state) and very low (DOWN state) cortical input. Moreover, recent studies emphasize that very similar network dynamics underlie the depolarized cortical states of waking and sleep [25]. This is important because it implies that what we see during slow wave UP states may be extended to the persistently depolarized state seen in wakefulness. Thus, instead of looking at the slow waves of anesthesia only because of their resemblance with natural sleep, we can think of them as a tool to study the behavior of networks during episodes of sustained cortical activity. As a tool, the slow wave offers advantages from a statistical perspective (as we get lots of repetitions of the same phenomenon in a short time) and from the fact that each episode of sustained activity is bounded by almost complete silence. Kasanetz et al. (2008) exploited these advantages to demonstrate that corticostriatal channels are not fully segregated. More specifically, by recording the traveling slow wave in three cortical areas simultaneously with the membrane potential in one MSNs, we established that (i) the cortical area that leads the traveling wave induces phase advances in UP state onset in functionally unrelated striatal territories; (ii) the cortical area that closes the wave extends the UP state in unrelated striatal territories (Fig. 9.1). This first demonstration of cross-talk between corticostriatal channels was possible thanks to the alternating episodes of silence and firing that underlie the slow waves. However, the message is that cross-talk is an integral component of the network depolarized state during which influences are spread over wide striatal territories.

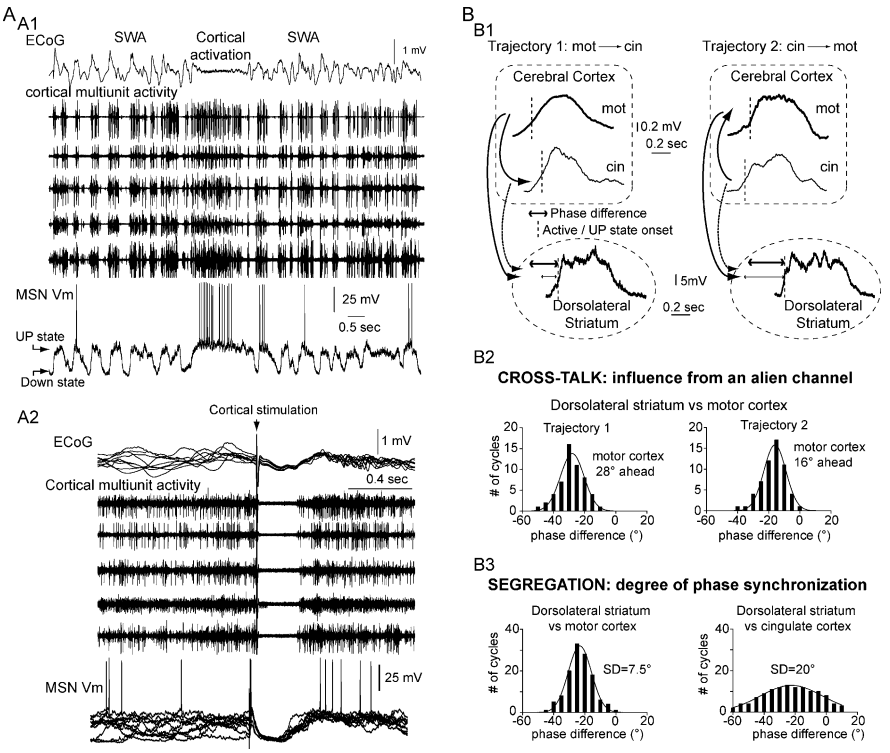

Fig. 9.1 **A**. Correlated cortical and striatal activities. Simultaneous recording of the electrocorticogram (ECoG), multi-unit activity (MUA) in the deep layers of the motor cortex, and the membrane potential of a MSN located in the dorsolateral striatum, under urethane anaesthesia in a normal rat. **A1**. During cortical slow wave activity (SWA), transitions between UP and DOWN states in MSNs are coincident with episodes of firing and silence in cortical ensembles. When the ECoG looks activated, cortical ensembles exhibit persistent activity and MSNs show a persistent UP state. **A2**. A single electrical pulse delivered to the cerebral cortex turns off cortical firing and stops striatal UP states [23]. After a pause (DOWN state), the cortex and MSNs restart synchronously. Several trials of stimulation are displayed, aligned at the time of stimulation. **B**. Segregation and cross-talk in corticostriatal circuits. Drawings depicting predictions about how the phase relationship between a MSN located in the dorsolateral striatum and the ECoG in its more functionally related cortical area (motor cortex: mot) should change depending on the trajectory followed by the slow wave across the cortex (**B1**). If corticostriatal channels were completely segregated, this phase difference should be the same regardless of slow wave trajectory. Instead, Kasanetz et al. (2008) demonstrated that when the slow wave recruits a non-matching cortex first (cingulate cortex: cin), this phase difference shortens, indicating that activity in the cingulate cortex contributes to driving the UP state (**B2**). Cross-talk is bi-directional (the motor cortex influences UP state onset in the medial striatum). This does not mean that there is no corticostriatal segregation. Functional segregation was demonstrated by showing that striatal UP states are better synchronized with slow waves in the functionally related cortical area (**B3**)

Spontaneous Activity Downstream the Striatum

The classical model of Parkinson's disease pathophysiology proposed that an imbalance between the activities of MSNs projecting through the direct and indirect pathways is responsible for all functional changes downstream the striatum and for the main clinical manifestations of patients [1, 2]. Although it has also been posited that dopamine depletion causes an increase in the gain of the cortico-subthalamic pathway and adaptations within the subthalamic nucleus (STN) and globus pallidus (GP) [8, 26, 27], evidence in this regard is scarce compared with the already well-established striatal imbalance (see the chapter by Ballion et al.).

Evidence of MSN dysfunction in rat parkinsonism was provided by Tseng et al. [20], who showed that MSNs reach a more depolarized membrane potential during UP states and then have more chances of firing action potentials during each slow wave. As a result, there are more MSNs encoding the main frequency of the slow wave in their spike trains. Importantly, as the UP state is required for firing, it is seen as a gating mechanism that enables the transmission of cortical information through MSNs [16]. In addition to providing in vivo evidence for a role of dopamine in controlling the flow of cortical activity through the striatum, Tseng et al. proposed (for the first time) that this mechanism could explain the oscillations emerging downstream in the basal ganglia circuit after dopamine depletion (see also [9]). More recently Mallet et al. [28] found a way to functionally identify MSNs as belonging to the direct or indirect pathway and demonstrated unequivocally that those MSNs projecting to the GP are spontaneously hyperactive during slow waves in rat parkinsonism.

Further studies by Zold et al. [29, 30] examined how nigrostriatal lesion impact on coordination between spontaneous activity in the motor cortex, striatum, and GP in urethane-anesthetized rats. GP neurons behave as pacemakers in vitro [7] but are modulated by cortical activity in vivo. In normal rats this modulation consists of increased firing during the "active" part of the slow wave [31] (see [32] for the effects of different anesthetics). Zold et al. demonstrated for the first time that after an extensive nigrostriatal lesion, most GP neurons show the opposite relationship with cortical slow waves, that is, reduced firing coupled to striatal ensembles activation during the active part of the wave (see also [33]).

At first sight, the significance of these findings to Parkinson's disease could be difficult to understand. But, from a mechanistic point of view, our findings have several implications. Cortical activity is conveyed to the GP mainly through two pathways, the indirect pathway through MSNs, which is inhibitory; and the cortico-STN-GP pathway, which is excitatory. Zold et al. [29, 30] proposed that dopamine depletion shifts the balance between these two pathways, from dominant STN input in control conditions to dominant striatal input in parkinsonism (Fig. 9.2). Importantly, we demonstrated that, also during episodes of cortical activation, GP neurons are more likely to show

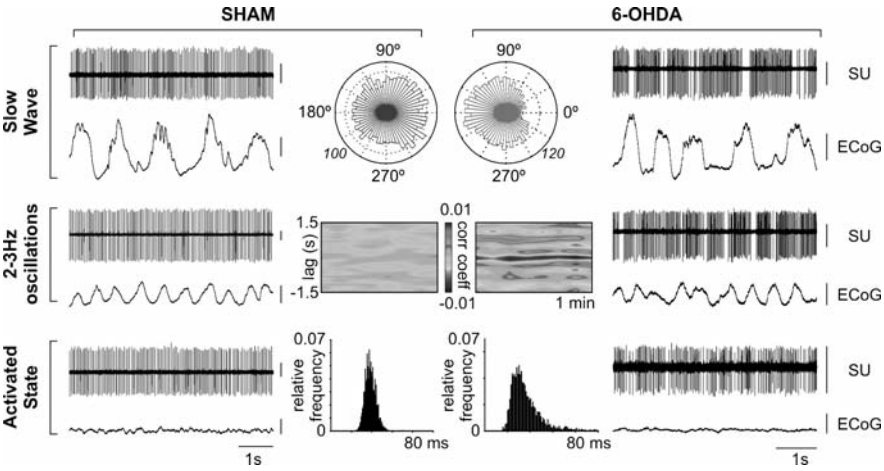

Fig. 9.2 Parkinsonian rats exhibit similar changes in GP activity across different cortical activity states. GP neurons' spontaneous activity (SU) recorded under different cortical local field potential (ECoG) states in control and dopamine depleted rats. Slow wave activity. Most GP neurons increase their firing rate during periods of high cortical input in control rats. This is illustrated in a circular distribution of the phases of the ECoG at the time of occurrence of GP spikes. In the graph, 0° represents the maximum of the slow wave, which in our recordings correlates with high cortical firing, and the radial axis (italics) is the number of spikes. In dopamine-depleted rats, many GP neurons show the opposite phase relationship with the slow wave, decreasing their firing rate during the period of high cortical input. 2–3 Hz oscillations. GP neurons also show significant coupling to cortical activity when the cortex is oscillating at higher frequencies (2–3 Hz) in dopamine depleted rats, showing pauses in correlation with ECoG oscillations. This is also illustrated in time-evolving cross-correlograms. Activated state. Resting GP firing pattern is also abnormal during episodes of cortical activation in rats with severe nigrostriatal lesion. GP neurons show more variable interspike interval distributions and are more likely to show pauses in these rats. Note that GP neurons in control rats display regular firing when the cortex is activated as can be seen in the narrow intersipke interval distribution. ECoG bar: 0.2 mV. SU bar: 0.5 mV

pauses in spontaneous firing in dopamine-depleted rats, suggesting that the aberrant inhibitory mechanism operates whenever the cortex is activated [29]. Indeed, by splitting the slow wave in its silent and active parts, we showed that the pauses do not occur when the cortex is silent, and that the distribution of interspike intervals is abnormally skewed to the right (because of the long intervals) when the cortex is active. Moreover, this positive skewness occurs regardless of whether the period of activity was limited to a slow wave or it is sustained. This adds to the view that cortical activation and the depolarized microstates embedded within the slow waves can raise similar dynamics in the basal ganglia.

Taken as a whole, the above supports that an increased gain of the corticostriatopallidal axis drives functional changes in spontaneous activity in the GP in experimental parkinsonism. Rather than a decrease in the average firing rate, as predicted by the classical model, the main change induced by dopamine depletion

is a higher probability of showing pauses in firing when the cortex is active. The intervals between pauses can be shaped by the cortex and emerge as an oscillation, as in the slow wave state, or be poorly organized and be seen as an irregular firing pattern, as occurs during cortical activation. It seems likely that other patterns of cortical input will shape the pauses differently, resulting in other oscillatory patterns in the parkinsonian GP (Fig. 9.2) [30]. It is also likely that the GP drives the pathological slow oscillations in the basal ganglia output nuclei [34], since they show exactly the opposite phase relationship [33], as it would be expected from the fact that GP output is GABAergic

However, our main conclusion is at odds with the view that the STN itself or an increased gain of the cortico-subthalamic pathway underlies oscillations and synchrony in parkinsonism [7, 26]. This hypothesis is supported by in vivo studies showing that STN lesions reinstate a normal pattern of activity in the GP of dopamine-depleted rats [35]. The possibility remains that the STN could somehow allow the induction of pauses in the GP by the increased striatopallidal input. However, it should also be considered that an STN lesion may induce adaptations in the cortex and basal ganglia by multiple mechanisms, for instance, by stopping the flow of information through a closed loop involving the striatopallidal neurons. Thus, it cannot be taken for certain from lesion studies that the STN induces changes in GP activity through direct connections. On the other hand, our conclusion is based on the assessment of functional connectivity between the cortex, striatum, and GP and this cannot be taken as evidence of causal associations [36]. In the end, studies of functional connectivity should be combined with local drug treatments in the striatum to clearly establish whether information flow through hyperactive striatopallidal neurons is the actual cause of the pauses in the GP.

Electrical Stimulation as a Tool to Study the Dynamic Activation of Trans-striatal and Trans-subthalamic Pathways

Despite the effort spent in clarifying the mechanisms underlying changes in spontaneous activity in Parkinson's disease, there is little theoretical work on how these changes may cause the clinical manifestations of the illness. The classical model proposed that akinesia stems from an increased basal ganglia output that maintains the motor thalamus under tonic inhibition. However, it is not clear if the resting firing rate of neurons in the output nuclei is actually increased in parkinsonism. In addition, our recent work [29] indicates that changes in resting activity appear only after large nigrostriatal lesions and therefore may not contribute to early motor impairment (see [37] for monkeys). Still, this does not lessen the importance of understanding how changes in resting activity arise, since they may contribute to the late signs of the disease, which are in fact the more disabling ones.

On the other hand, there is plenty of theoretical and experimental work on how basal ganglia activity is shaped during movement. Models converge on the view that the inhibitory trans-striatal direct pathway triggers movement by disinhibiting specific sets of thalamo-cortical neurons [38]. The trans-striatopallidal and trans-subthalamic pathways seem to carry excitatory signals to the output nuclei which limit in time and space the inhibitory effect of the direct pathway, precluding the execution of competing movements and shaping movement sequences [11, 39]. In this context, it has been posited that an imbalance favoring the action of the trans-striatopallidal and trans-subthalamic "movement-arresting" pathways may impede movement. This would show as a wide excitation and a smaller proportion of output nuclei neurons displaying phasic inhibition during voluntary movement, as has been recently demonstrated in parkinsonian monkeys by Leblois et al. [40] (see [41, 42] for less direct evidence coming from passively moving monkey's joints). However, it is difficult to ascertain from these experiments in behaving animals if (i) parkinsonian and normal movements can be readily compared; (ii) if the reported changes in activity stem from sensory feedback or are a reflection of corrupted action instructions; (iii) and if the mechanisms underlying these changes involve modifications in the gain of specific pathways.

The question remains, how can an anesthetized animal help us to understand the behavior of the trans-basal ganglia pathways during movement? Several studies have already described that delivering single electrical shocks to the motor cortex activates all trans-striatal pathways, inducing a complex response in the GP and the output nuclei. The sequential components of these responses have been related to the conduction time of the different pathways [43–47]. Moreover, by stimulating two different cortical sites it is possible to study spatial integration at the level of single basal ganglia neurons. All this can be done by minimizing any sensory feedback, including that related to movement, which is usually not seen when low stimulation currents are delivered to the motor cortex under urethane anesthesia.

Evoked Activity Downstream the Striatum

Curiously, the effect of nigrostriatal lesions on the response of basal ganglia neurons to motor cortex stimulation has not been studied in detail until recently. On the input side of the circuit, Mallet et al. [28] found that, in addition to being spontaneously hyperactive, striatopallidal MSNs are over-responsive to motor cortex stimulation in dopamine-depleted rats. Conversely, the direct pathway is dramatically depressed. This implies that the striatal imbalance concerns both spontaneous activity and the processing of phasic inputs.

In the SNr, the main output nucleus of the rodent basal ganglia, motor cortex stimulation causes an inhibition, which is mediated by the direct pathway. In control conditions this inhibition is delimited by an early excitation ($<$10 ms latency), caused by the trans-subthalamic pathway, and a late excitation ($>$20 ms latency), which involves the multi-synaptic indirect

pathway and, perhaps, reverberations in the GP-STN network. Consistent with the view that dopamine depletion favors the "movement-arresting pathways", Belluscio et al. [48] have recently demonstrated that inhibitions are replaced by sustained excitations in rats with chronic nigrostriatal lesion

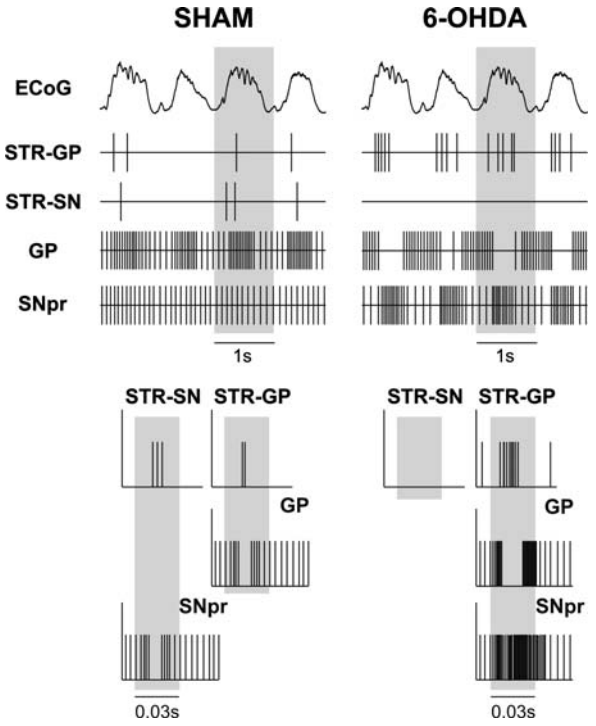

Fig. 9.3 Role of the indirect pathway in Parkinson's disease pathophysiology. Illustrations representing the changes induced by dopamine depletion in basal ganglia resting and evoked activity. **A**. Relationships between the spontaneous activity of the motor cortex, striatum, GP and SNr, during cortical slow wave activity in control and parkinsonian rats. In the striatum, the lack of dopamine induces an imbalance between the direct and indirect pathways. Striatopallidal (STR-GP) neurons become hyperactive and striatonigral (STR-SN) neurons depressed. In the GP, pacemaker activity is modulated by cortico-subthalamic input in controls, but cortico-striatal input becomes more important in parkinsonism, inducing pauses in firing when the cortex is active during slow waves. Consistent with the changes observed up-stream, SNr neurons show slow oscillations in dopamine depleted rats. **B**. Activity evoked by cortical stimulation in the striatum, GP and SNr. Dopamine depletion induces an imbalance in the processing of phasic inputs in the striatum, which favors transmission through striatopallidal neurons. Accordingly, GP neurons become over-responsive to cortical stimulation showing more inhibitory responses. The time window during which cortical stimulation normally induces inhibition in the SNr via the direct pathway is occupied by a premature late excitation in parkinsonism. Premature late excitations occur at the time of the enhanced GP inhibitions. Overall, if we adjust the spatial and time frames we are using to analyze the data, these observations can be accounted for by the striatal imbalance model

(Degos et al. [49] reported a somewhat similar effect of an acute neuroleptic treatment). Moreover, we found that intrastriatal administration of dopamine receptor agonists (either D1- or D2-type selective) reduce and delay the pathological excitations, allowing, in some instances, the appearance of an inhibition. This study provides evidence supporting the idea that striatal dopamine shapes basal ganglia output in response to phasic cortical input and suggests that the striatal imbalance drives "premature late excitations" in the output nuclei.

Evidence supporting a contribution of the indirect pathway in driving an altered basal ganglia output in parkinsonism came from studies of GP responses to motor cortex stimulation [29]. Owing to the opposite influences the "movement-arresting" pathways exert on the GP, motor cortex stimulation elicits an early excitation (via the STN) followed by an inhibition (via the striatopallidal MSNs) in GP neurons (Fig. 9.3). Thus, GP responses would depend on the relative gain of those pathways. Zold et al. found that GP neurons are several fold more likely to respond to stimulation of a single cortical focus after dopamine depletion, owing to an increased number of inhibitions, in rats with large nigrostriatal lesions. (We also saw increased late excitations, which probably reflect rebound disinhibition of STN activity.) These changes also lead to overlapping target fields of cortical stimulation sites, that is, increased convergence of cortical inputs in the GP.

Together, these results suggest that severe nigrostriatal degeneration increases the gain of the striatopallidal axis for phasic inputs, resulting in disinhibition of GP targets. Importantly, in addition to the STN, GP projections target the proximal dendrites and soma of SNr neurons [50]. Moreover, the timing of GP inhibition matches well that of the "premature late excitations" seen by Belluscio et al. in the SNr (Fig. 9.3). However, there is a piece missing in this puzzle. Nobody has still reported how STN neurons respond to cortical stimulation after dopamine depletion. This would help clarify the relative contributions of the trans-subthalamic and trans-striatopallidal pathways in driving the increased basal ganglia output.

Conclusions

The alterations seen in GP's spontaneous activity during the active microstates that underlie slow waves look similar to those seen during prolonged cortical activation. Because the alterations are not seen when the cortex is silent, we propose a common mechanism involving cortical input. When cortical activity oscillates, it patterns temporally the pauses, raising oscillations in the GP, but pauses can also emerge as non-oscillatory patterns. Neurons in the SNr show changes opposite in phase to those seen in the GP. The easiest possible explanation for this is that striatopallidal neurons loose the ability to filter out incoming signals and thus end up reflecting the spontaneous dynamics of the cortical

depolarized state. Theories about how abnormal spontaneous activity contributes to Parkinson's disease need to consider what is the meaning of internal activity states [51] and why should the basal ganglia filter them out.

After extensive dopamine depletion the basal ganglia target fields of cortical stimulation sites increase in size and overlap. In the GP, this is due to increased inhibitory effects and the delayed excitations that follow them. In the SNr, an excitation dependent on striatal dopamine deficiency takes place at the time of the supposedly maximal inhibitory action of the direct pathway. In the context of theoretical models on how basal ganglia output encodes action instructions [11, 39], this could mean that the spatial and temporal delimiting actions of the indirect pathway are enhanced to the point of erasing the main action command.

Overall, recent findings match the predictions of the striatal imbalance model. Perhaps the basic assumptions of the "static" model are correct and we just need to adapt the spatial grid and time frame we have been using to read it (Fig. 9.3).

Several questions remain open however. Partial nigrostriatal lesions inducing clear motor deficits are not associated with obvious changes in spontaneous activity, nor with increased inhibitory responses to cortical stimulation, in the GP. Instead, GP neurons show over-excitation with loss of spatial segregation following cortical stimulation in rats with partial nigrostriatal damage. This could mean that the enhancement of the indirect pathway occurs late during disease progression, contributing to the profound akinesia and other incapacitating clinical manifestations seen at advanced stages of the illness. At early stages, bradykinesia could be related to more subtle changes in spatial integration and a rise in the gain of the cortico-subthalamic pathway [29]. This remains to be proven, however, as there is no evidence to support that there is an increase in the gain of the cortico-subthalamic pathway in parkinsonism.

Recent studies in parkinsonian primates show profound alterations of basal ganglia output during voluntary movement, including an increase in the ratio of excited over inhibited neurons during movement [40]. Also, they show a marked synchronization of cortical and basal ganglia activities [52, 53]. The above rat studies provide grounds to understand the origin of these changes at the circuit level. More research at this level is needed to assess the role of specific pathways in shaping functional connections as disease progresses.

A recent computational modeling study by Leblois et al. [10, 40] could serve as a framework for future work. It proposes that action selection results from competition between closed cortex-basal ganglia feedback loops that favor movement (direct pathway or positive feedback loop) and arrest movement. The model predicts that partial dopamine depletion impairs action selection because of a loss of segregation and that oscillations emerge only after large simulated depletions. The model also stresses the role of the cortico-subthalamic pathway as the main negative feedback loop. Recent findings from our labs suggest that the indirect pathway could also play an important role, especially in advanced stages of the illness, contributing to the appearance of oscillations.

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Chapter 10
The Corticostriatal Transmission in Parkinsonian Animals: In Vivo Studies

Bérangère Ballion, Nicolas Mallet, Catherine Le Moine, Mario Gustavo Murer, and Francois Gonon

Introduction

The striatum is the main input structure of the basal ganglia (BG) [1]. Because it receives massive excitatory inputs from the cortex and mainly projects to deeper BG nuclei, the treatment and transmission of cortical inputs by striatal neurons represent a crucial step in the cortex–BG–thalamus feedback loops. Moreover, the striatum is the brain area that is the most densely innervated by dopaminergic terminals. Because Parkinson's disease is characterized by a severe degeneration of dopaminergic neurons, it is crucial to understand how the dopamine (DA) depletion affects the transfer of information from the cortex to the BG via the striatum. In this chapter we will review recent in vivo studies addressing these questions. However, in vitro studies investigating the corticostriatal transmission are not deeply discussed here because others review them elsewhere in this book.

Cortical Inputs to Striatonigral and Striatopallidal Neurons in Intact Animals

The Direct and Indirect Striatal Output Pathways

In the rat, medium-sized spiny neurons (MSNs) represent 95% of the striatal neurons. They are GABAergic, project outside the striatum and are organized into two distinct pathways: striatonigral neurons form the direct pathway and striatopallidal neurons form the first element of the indirect pathway [1]. In rodents, striatonigral neurons send their main axon either to the substantia nigra pars reticulata (SNr), to the entopeduncular nucleus, or to both, where

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they form a richly arborized terminal plexus. Most striatonigral neurons also send a few poorly branched collaterals to the external part of the globus pallidus (GPe), whereas striatopallidal neurons densely innervate the GPe but do not send axons to other downstream BG nuclei [2–4]. Striatonigral and striatopallidal neurons also differ in the neuropeptide they express: substance P and enkephalin, respectively [1]. Colocalization of substance P and enkephalin is low (3–4%) in adult rats but more common (8%) in younger (3–4 weeks old) rats [5].

Until recently the anatomo-functional approach using immediate early gene (IEG) expression presented a decisive advantage over electrophysiology to study in vivo striatal function and dysfunction. Indeed, the former approach makes it possible to identify individual MSNs according to the expression of their specific markers [6–11]. However, we recently showed that extracellular recording of single MSNs can be combined with antidromic SNr stimulation to identify striatonigral neurons [12]. We also showed that MSNs exhibit electrophysiological characteristics clearly distinct from cholinergic and GABA interneurons [12]. Moreover, we showed that striatal neurons, which exhibit electrophysiological characteristics of MSNs, but which do not respond to an accurate antidromic stimulation of SNr, can be considered as striatopallidal neurons with a high degree of certainty [12, 13].

Segregation of D1 and D2 Receptors in Striatonigral and Striatopallidal Neurons: Recent Evidence

Several lines of evidences suggest that DA modulates the response of MSNs to cortical inputs. However, the effects of DA are mediated by five types of dopaminergic receptors belonging to two families: the D1 family (D1 and D5) and the D2 family (D2, D3 and D4) [14]. In the rat dorsal striatum, MSNs only significantly express D1 and D2 receptors, which are positively, and negatively, coupled to adenylate cyclase, respectively [14]. Initial studies with in situ hybridization showed that striatonigral neurons mainly express D1 receptors whereas striatopallidal neurons mainly express D2 receptors [6–8]. The use of more sensitive cRNA probes confirmed the prominent segregation of D1 and D2 receptors in striatonigral and striatopallidal neurons, respectively [15]. In addition, other studies combining immunohistochemistry with electron microscopy also supported the segregation view [16–18]. However, this view has been initially questioned by a study using RNA analysis on single cells [19], and more recently by one study using immunohistochemistry with confocal microscopy and showing that "virtually all striatal neurons" contain D1 and D2 receptors [20]. Nevertheless, this important controversy regarding the segregation of D1 and D2 receptors has been definitely clarified by gene targeting. Indeed, when the expression of the diphtheria toxin is linked to the D1 receptor gene, striatonigral neurons are completely lesioned whereas striatopallidal neurons are spared [21]. Moreover, bacterial artificial chromosome (BAC) transgenic

mice were generated to link a green fluorescent protein (GFP) either to D1 or D2 receptor genes. In these mice, MSNs expressing the D2 receptor also express enkephalin, but not substance P, and project to the globus pallidus but not to the substantia nigra pars reticulata (SNr), whereas MSNs expressing the D1 receptor also express substance P, but not enkephalin, and heavily project to the SNr [3, 4, 22].

In conclusion, in the rodent striatum, most striatonigral neurons only express the D1 receptor and most striatopallidal neurons only express the D2 receptor. Less than 5% of MSNs may co-express both genes [15]. In the non-human primate this segregation is also prominent [23] although it may be less than in rodents [24]. However, interestingly enough, this segregation is not affected by DA depletion and by subsequent chronic treatment with L-DOPA [24].

Cortical Inputs to Striatonigral and Striatopallidal Neurons

Retrograde tracer studies showed that any restricted striatal area always receives convergent inputs from multiple cortical areas [25, 26]. In the rat, two types of cortical projection neurons innervate the striatum. Cortical neurons that project inside the telencephalon (IT) bilaterally innervate the ipsi and contralateral striatum whereas cortical neurons that sent their main axon into the pyramidal tract (PT) only innervate the ipsilateral striatum. PT neurons are located in layer V of the cortex, whereas IT neurons are located in layer III and layer V [27–31]. One anatomical study combining retrograde tracer with immunohistochemical identification of striatonigral and striatopallidal dendrites suggested that PT neurons mainly contact striatopallidal neurons whereas IT neurons preferentially synapse on striatonigral neurons [18]. However, a previous study showed that IT neurons equally contact D1-positive and D2-positive neurons [16].

Electrophysiological tests using antidromic stimulations can be used to identify PT versus IT neurons [28, 32]. In the intact rat anesthetized with urethane, IT and PT corticostriatal neurons exhibit similar spike waveform [28] and, according to a previous study [28], similar discharge rate. However, we observed, with a much larger number of neurons, that PT neurons exhibit a significantly ($p < 0.01$, unpaired t test) higher discharge rate (2.13 ± 1.78 Hz, mean $\pm$ SD, $n = 94$) than IT neurons (1.47 ± 1.14 Hz, mean $\pm$ SD, $n = 66$) [32]. Moreover, the conduction velocity of PT neurons is about 4.6 times faster than that of IT neurons [28, 32].

Using our electrophysiological approach we tested the attractive possibility that IT and PT neurons preferentially contact striatonigral and striatopallidal neurons, respectively [32]. Two predictions result from this hypothesis and from the specific characteristics of IT versus PT neurons. First, because IT neurons are largely bilateral whereas PT neurons only innervate the ipsilateral striatum, electrical stimulation of the contralateral cortex should be more potent on

striatonigral than on striatopallidal neurons. Second, because the conduction velocity of PT neurons is 4.6 times faster than that of IT neurons, striatopallidal neurons should respond earlier than striatonigral neurons to ipsilateral stimulation. However, our data did not support these predictions [32]. Instead, our results support the following interpretations. First, IT neurons equally excite striatonigral and striatopallidal neurons. This functional conclusion is in conflict with the prediction made by Lei et al. on the basis of their anatomical data [18]. However, with similar tracer injections others showed that IT synapses contact similar number of striatonigral and striatopallidal dendrites [16]. Second, compared to IT neurons, PT neurons weakly contribute to excitation of MSNs. This view is consistent with anatomical studies of individually labeled IT and PT neurons [28, 33]: "the axonal arborizations formed in the striatum by PT cells are small and sparse compared with IT cells." Moreover, this is in line with the fact that the primate striatum does not receive any collateral from neurons of the motor cortex, which project to the brain stem [34].

Spontaneous MSN Discharge Activities Generated by Cortical Inputs

In anesthetized rats the dominant state of the electroencephalogram (EEG) recorded at the level of the motor cortex is often named "cortical synchronization" and is characterized by slow waves of large amplitude at ~1 Hz. This rhythmic cortical field potential is caused by the rhythmic discharge activity of almost all cortical neurons [13, 35, 36]. Regarding IT neurons, which provide the main excitatory input to MSNs, this discharge activity is about 1.5 Hz on average [32]. In the same experimental conditions MSNs are either completely silent or discharge at a much lower rate [13]. Indeed, on average, striatonigral are less spontaneously active than striatopallidal neurons (0.03 and 0.15 Hz, respectively) [13]. This low rate of firing transmission from the cortex to striatum does not mean that, during slow wave state, corticostriatal neurons do not influence MSNs. In fact, during slow waves, the membrane potential of all MSNs oscillates between a hyperpolarized and a depolarized state and MSNs only discharge during their depolarized state [37]. These MSN oscillations are caused by corticostriatal inputs [37] and generate a striatal oscillatory field potential, which closely follows the cortical waves with a delay compatible with a monosynaptic transmission [12, 36]. Disruption of the cortical slow wave activity, either spontaneously occurring or induced by electrical stimulation of the mesopontine tegmentum, also disrupts the oscillations of MSN membrane potential [38]. In this desynchronized state, the MSN membrane potential is close to that observed during rhythmic depolarization [38], but the MSN discharge activity remains much lower than that of corticostriatal neurons [12].

In anesthetized animals, the discharge activity of MSNs is always very low compared to that of corticostriatal neurons. Anesthetics certainly contribute to

depress the corticostriatal transmission. Indeed, urethane or chloral hydrate anesthesia contributes to inhibit the discharge activity of MSNs during slow waves [39, 40]. However, recordings in freely moving rats show that the MSN discharge activity is very irregular and still low in resting conditions (0.63 Hz on average) [41]. When cortical neurons and MSNs are simultaneously recorded, the mean discharge rate of the former (2.13 Hz) largely exceeds that of the latter (0.78 Hz) [42]. Finally, in rats and monkeys, MSNs are much less active during resting conditions than during conditioned behavior, either during the preparation of movement or during movement [43–45]. All together, the discharge rate of MSNs is not linearly related to that of corticostriatal neurons: it is very irregular and strongly related to movement.

MSNs Activities Evoked by Cortical Stimulation

To test the efficiency of the corticostriatal transmission in anesthetized rats several groups have used electrical stimulations of the cortex. Anatomo-functional studies measuring the expression of IEG as an index of activation of identified striatal neurons showed that cortical stimulation either similarly stimulated striatonigral and striatopallidal neurons [46] or preferentially stimulated striatopallidal neurons [47, 48]. This approach also showed that the contralateral cortical stimulation is almost as effective as the ipsilateral one [48] and that parvalbumin-positive GABA interneurons are much more responsive to cortical stimulation than MSNs [47]. We were the first to study the effects of cortical stimulation on the spike response of identified MSNs [12]. We observed that ipsilateral cortical stimulation is equally efficient on striatonigral and striatopallidal neurons [12, 32]. However, among MSNs that respond to both ipsi and contralateral stimulation, striatopallidal neurons are slightly more responsive than striatonigral neurons [32].

Feedforward Inhibition of MSNs by FS Interneurons

In the striatum, the best-characterized GABAergic interneurons are those that express parvalbumin [49]. These interneurons are named “fast spiking” (FS) because their spike waveform is briefer and sharper than any other types of striatal neurons either in vitro [50] or in vivo [12, 41]. In vitro electrophysiological studies show that, in the rodent striatum, the inhibitory synapse formed by FS interneurons on MSNs is much stronger than the collateral inhibition between MSNs [51] and is very rapid (MSN IPSPs peak $\sim$5 ms after the FS action potential) [50].

We distinguished FS interneurons from any other neuronal type in the anesthetized rat by means of three criteria: (1) their briefer spike waveform, (2) their high-frequency bursting response to supra-threshold cortical

stimulation and (3) the fact that they are more responsive to the first pulse in a pair of cortical stimulations applied 100 ms apart [12]. This third feature is due to the fact that the spontaneous cortical activity is completely blocked 100 ms after a cortical stimulation. Because the spike response of FS interneurons to

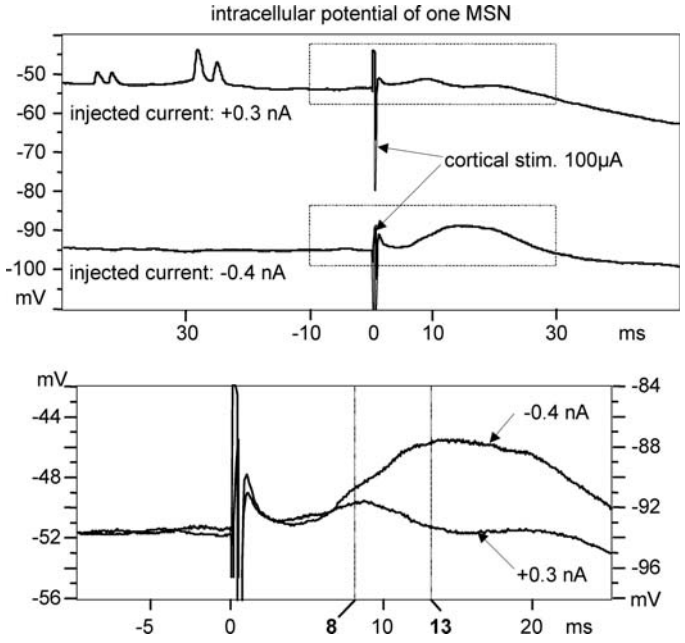

Fig. 10.1 GABA-induced synaptic responses evoked in vivo by cortical stimulation. Cortical stimulation induces in MSNs both EPSPs via direct corticostriatal synapses and IPSPs mediated by FS interneurons. To reveal these IPSPs the experimental conditions were selected to maximize the influence of FS interneurons: recording was performed during the state of cortical desynchronization and the stimulating current was adjusted to a low level just sufficient to evoke spike responses in the majority of FS interneurons. Intracellular recordings were performed with sharp electrodes (50–80 MΩ) filled with potassium acetate (3 M). In these experimental conditions the reversal potential of Cl^- is close to –60 mV. Therefore, GABA depolarizes MSNs when they are maintained in an hyperpolarized state by intracellular injection of a hyperpolarizing current (–0.4 nA) and hyperpolarizes MSNs when they are maintained in a depolarized state by intracellular injection of a depolarizing current (+0.3 nA). Therefore, it is likely that the difference between recordings obtained in both states reveals GABA-induced effects. The traces shown in the upper part of the figure correspond to the averaging of 20 consecutive recordings obtained in both conditions when cortical stimulations were applied every 3 s. In the depolarized conditions spontaneous spikes sometimes occur before the cortical stimulation and were averaged in the trace. The lower part of the figure shows an enlargement and a superimposition of both traces (the *left*and *right*scales in mV correspond to recordings obtained with injections currents at +0.3 and –0.4 nA, respectively). Notice that both traces start to diverge 8 ms after the stimulation and that their difference reaches its maximum ~13 ms after the stimulation. Similar data were obtained from four additional MSNs. Therefore, these kinetics of the synaptic transmission from FS interneurons to MSNs are similar to those observed in vitro and compatible with feedforward inhibition

cortical stimulation is facilitated when it occurs during a period of spontaneous cortical activity, their response to the second pulse is hindered compared to that evoked by the first pulse in a pair at 100 ms interval [12].

We observed that FS interneurons are much more responsive than MSNs to cortical stimulation [12]. This difference is even more obvious when the EEG shifts from the slow wave state to cortical desynchronization. More generally, experimental conditions that favor the spike discharge of FS interneurons invariably hinder that of MSNs [12]. We concluded that FS interneurons provide a potent feedforward inhibition on MSNs. This feedforward inhibition is possible because FS interneurons respond earlier than MSNs to cortical stimulation by ~5 ms [12], and because the synapse formed by FS interneurons on MSNs is fully activated within 5 ms as shown in slices [50] and with in vivo experiments (Fig. 10.1).

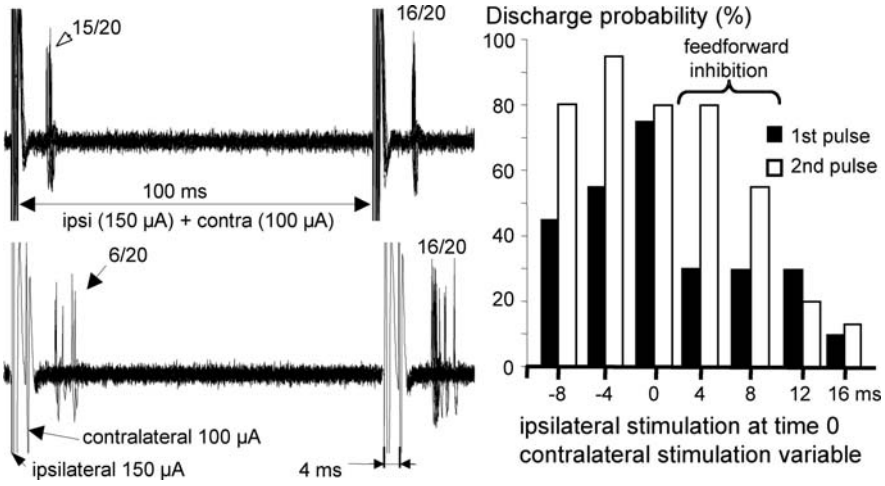

Fig. 10.2 Spike responses of one striatonigral neuron to simultaneous or asynchronous electrical stimulations of the ipsilateral and contralateral cortex. Pairs of electrical stimulations at 100 ms intervals were applied every 3 s. For every condition the discharge probability was estimated from the spike responses observed in 20 tries. The stimulating currents were adjusted so that either the ipsi or the contralateral stimulations were subthreshold for this neuron when applied alone. When both stimulations are applied simultaneously (*left top*), the discharge probability is high regarding both the first and the second pulse. When the ipsilateral stimulation preceded the contralateral by 4 ms (*left bottom*) the discharge probability was strongly decreased only regarding the first pulse. Because the ipsilateral and contralateral stimulations were adjusted at 150 and 100 µA, respectively, it is likely that the former was more potent to evoke spike responses in FS interneurons. Therefore, when the ipsilateral stimulation preceded the contralateral stimulation for 4 or 8 ms, the spike responses of this striatonigral neuron to the first pulses were inhibited by FS interneurons. Notice that, because the response to the second pulses is much less affected by feedforward inhibition, the addition of the contralateral stimulation 4 and 8 ms after the ipsilateral stimulation is more effective regarding the second pulse than for the first

We showed that FS interneurons preferentially inhibit MSNs exhibiting the weakest sensitivity to cortical stimulation [12]. Thus, this feedforward inhibition filters cortical information effectively transmitted by MSNs. Moreover, we showed that this inhibition also narrows the time window of the MSN responses evoked by cortical stimulation [13]. Therefore, feedforward inhibition might limit the time window for the summation of multiple asynchronous inputs as already shown in the cerebellum [52]. We performed experiments to test in the striatum this important function of FS interneurons. As illustrated in Fig. 10.2, feedforward inhibition is able to control the additive effects of two distinct cortical inputs by inhibiting asynchronous inputs.

Effects of the Dopaminergic Depletion on the Corticostriatal Transmission

Effects of DA Depletion on Striatonigral and Striatopallidal Neurons

Classical models of Parkinson's disease posit that the dopaminergic depletion imbalances the striatal activities: striatopallidal neurons are more active whereas striatonigral neurons are depressed [53–55]. This view has been supported by anatomo-functional studies [6, 9, 11]. Several in vivo studies have consistently shown that, on average, the spontaneous discharge activity of unidentified MSNs is enhanced in 6-OHDA-lesioned rats [40, 56–59]. We showed that the DA depletion strongly enhanced the spontaneous activity of striatopallidal neurons but depressed that of striatonigral neurons [13]. Our data are consistent with previous observations because the absolute increase in the spontaneous activity we observed in striatopallidal neurons greatly exceeded the decrease observed in striatonigral neurons. Regarding the responses evoked by cortical stimulation, previous studies reported an increase in the responsiveness of unidentified MSNs [60, 61]. In fact, we observed a prominent increase in the responsiveness of striatopallidal neurons but a pronounced decrease of striatonigral neurons [13]. It is likely that this latter effect may have escaped previous investigations because DA depletion renders these neurons both completely silent and poorly responsive to cortical stimulation.

Effects of DA Depletion on Feedforward Inhibition

In vitro studies have shown that FSIs are depolarized by DA via dopaminergic receptors of the D1/D5 type [62, 63]. Therefore, one might expect that the DA depletion should depress the discharge activity of FS interneurons. However, we observed that the lesion did not affect the spontaneous activity and

the response of FS interneurons to cortical stimulation [13]. Therefore, FS interneurons do not cause the striatal imbalance we observed in parkinsonian rats. However, we observed that the DA depletion narrows the time window of the spike response of striatonigral neurons and scatters that of striatopallidal neurons. Moreover, we showed that striatopallidal neurons tend to escape feedforward inhibition [13]. Therefore, FS interneurons do not cause, but worsen the striatal imbalance.

Origin of the Striatal Dysfunctions

Models of Parkinson's disease propose that the DA depletion disinhibits striatopallidal neurons via their D2 receptors and removes the facilitatory influence of D1 stimulation on striatonigral neurons [53–55]. However, changes in the activity of corticostriatal neurons might also contribute to striatal dysfunction. Indeed, we showed that in the cortex ipsilateral to a striatal DA depletion, the spontaneous activity of IT neurons is depressed by 51%, whereas that of PT neurons is not affected [32]. Because IT neurons provide the main functional input to both MSN populations, this cortical deficit cannot be the main cause of the striatal imbalance. However, it might participate in the depressed activity of striatonigral neurons. The hyperactivity of striatopallidal neurons might be due to a potent striatal mechanism, which overcomes the deficit of cortical input. Accordingly, in DA-depleted slices, the glutamatergic input is enhanced in a subpopulation of unidentified MSNs via a D2-sensitive mechanism [64].

Conclusion and Future Prospects

In agreement with the classical pathophysiological model of Parkinson's disease it is now clearly demonstrated that in the parkinsonian state, cortical inputs are more easily transmitted to the globus pallidus by striatopallidal neurons whereas the inhibitory control exerted by the direct pathway is strongly depressed. However, the mechanisms of this striatal imbalance are obviously more complex than suggested by the classical model. For example, the dramatic increase in the spontaneous discharge activity of striatopallidal neurons is in line with the model but, surprisingly, it is only moderately corrected (–28%) by a D2 agonist [65]. In contrast, the increase in the expression of zif-268 mRNA in striatopallidal neurons, which is caused by the DA depletion, is fully corrected by quinpirole [9]. This shows that the IEG expression in the striatum does not necessarily reflect parallel changes in the spontaneous discharge activity and emphasizes the need for in vivo electrophysiological studies.

The fact that D2 agonists effectively alleviate parkinsonism but only partially correct the increase in the spontaneous activity of striatopallidal neurons might also suggest that the mean rate of spontaneous discharge activity does not

adequately reflects the functional state of the striatal output pathways [65]. Dynamic changes in the discharge activities of the BG nuclei might be more relevant to BG functions and their alterations in Parkinson's disease as suggested by a theoretical model [66] supported by recent observations in monkeys [67, 68] and rats [65, 69]. For example, partial DA lesions in rats induce motor impairments and alter the responsiveness of GPe neurons to cortical stimulation but do not affect their spontaneous activity [69].

In conclusion, although models of Parkinson's disease propose, in the light of anatomo-functional studies [9, 11], that the combined stimulation of D1 and D2 receptors by L-DOPA or apomorphine effectively corrects the striatal imbalance, this is not necessarily true in terms of discharge activities. In order to fully investigate this question it will be necessary to study both the spontaneous discharge activity of identified MSNs and their dynamic responses to cortical inputs. We will also have to consider the role of GABAergic and cholinergic interneurons in controlling MSN activities.

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Chapter 11
Striatal Nitric Oxide–cGMP Signaling in an Animal Model of Parkinson's Disease

Anthony R. West, Stephen Sammut, and Marjorie A. Ariano

Nitric oxide (NO) is an important, yet understudied, gaseous regulator of neuronal activity in corticostriatal circuits. Numerous findings indicate that striatal NO signaling plays a key role in the regulation of short- and long-term synaptic plasticity [1, 2], protein kinase and protein phosphatase activities [3, 4], and gene expression [5]. Thus, disruption of striatal NO signaling cascades results in profound changes in behavioral, electrophysiological, and molecular responses to pharmacological manipulations of dopamine (DA) and glutamate transmission [6–8]. Most studies indicate that NO signaling facilitates neuronal activity via the activation of guanylyl cyclase (GC) (see [9] for review). It has been proposed that neuroadaptations in the NO–GC signaling cascade induced by DA depletion have an important role in the pathophysiology of Parkinson's disease (PD). We will review this evidence and describe our recent work using various in vivo electrophysiological and neuroanatomical techniques aimed at determining how unilateral partial DA lesions alter NO–GC signaling in striatal circuits. Our studies are beginning to unravel the complex neuroadaptations in NO–GC signaling cascades that emerge following modest depletion of striatal DA that potentially mimics the early stages of PD. These neuroadaptations are likely involved in the enduring changes in glutamatergic transmission and motor behaviors observed in parkinsonian animal models and patients with PD.

Parkinson's Disease

PD is a progressive neurodegenerative disorder characterized by resting tremor, rigidity, postural instability, and bradykinesia. PD usually develops spontaneously, later in life, following a preferential loss of the dopamine (DA) producing neurons of the substantia nigra (SN) [10]. The resulting disruption of

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DA neurotransmission in the nigrostriatal system and subsequent dysfunction of postsynaptic targets in the striatum is primarily responsible for the emergence of clinical symptoms [11]. The utility of DA replacement therapies for treating PD was discovered in the 1960s; however, these treatments may produce dyskinesias and other disabling side effects and do not appear to slow the progression of the disease [12]. Given the above limitations of using DAergic drugs like L-DOPA, intense efforts have been directed toward the development of novel therapeutic agents as monotherapies or as adjunct treatments with L-DOPA. [13] However, it is likely that a better understanding of the role of DA in regulating the activity of striatal neuronal subpopulations under normal and pathophysiological conditions is needed before more efficacious antiparkinsonian treatments will be developed.

Striatal Circuitry

The striatum receives a massive glutamatergic input from the entire neocortical mantle as well as subregions of the thalamus [14]. Sensorimotor information transmitted via these excitatory afferents is integrated in functionally coupled networks of striatal principal neurons and interneurons. Approximately 90–95% of striatal neurons are medium-sized spiny neurons (MSNs), which are easily identified by their unique physiological properties and dense spine-laden dendritic processes [15]. These projection cells contribute axons to one of two output pathways: the "direct pathway" or the "indirect pathway" [16]. MSNs of the direct pathway ("striatonigral" neurons) project directly to the basal ganglia output centers, the substantia nigra pars reticulate, and internal segment of the globus pallidus. Indirect pathway cells ("striatopallidal" neurons) connect the striatum with the output nuclei by way of the external segment of the globus pallidus and subthalamic nucleus. Simplified models of the structural organization of these pathways hypothesize that striatonigral neurons primarily express D1 receptors, whereas striatopallidal neurons contain mostly D2 receptors [14, 16]. Furthermore, the direct and indirect pathways function in opposition to one another [17].

At least three major subtypes of striatal interneurons have been distinguished based on morphology, physiological properties, and neurotransmitter localization [18]. These interneurons are aspiny and use acetylcholine, GABA, or GABA and NO as transmitters. Recent studies have shown that small networks of interneurons can exert powerful feed-forward influences over relatively large populations of MSNs (see [15] for review). Indeed, this feed-forward regulation has a considerable influence on the spike timing and short-term plasticity of MSNs recorded in vivo [2, 19, 20]. Importantly, numerous studies using brain slice preparations have shown that DA receptor activation exerts a powerful control over interneuron function and that this modulation has a critical impact on synaptic transmission and plasticity of MSNs (see [1] for review). Until recently the impact of DA depletion on the glutamatergic regulation of NO interneuron function and cGMP signaling has not been investigated.

Given the critical role for NO–GC signaling in regulating striatal output [1, 21], knowledge of how DA denervation modifies these processes is crucial for understanding the pathophysiology of PD.

Role of NO–GC Signaling in Motor Behavior

NO is formed via a NO synthase (NOS)-dependent process in which L-arginine and oxygen are converted into citrulline and NO. Although three distinct isoforms of NOS are expressed in the nervous system, neuronal NOS (nNOS) has a predominant role in modulating synaptic transmission. [22] Studies using unbiased stereological sampling techniques estimate that nNOS-containing interneurons make up less than 3% (~21,000 cells) of the total neuronal population of the striatum. [23] Because the axons of nNOS cells are extensive and project up to 1 mm from the soma, these cells are able to exert a tremendous functional impact on both blood flow and neural activity in large striatal subregions [24].

Numerous studies have reported that striatal nNOS interneurons play a key role in the generation of motor behavior (see [6] for review). Thus, systemic and intrastriatal administration of NOS and GC inhibitors decreases motor activity and induces catalepsy [25–28]. Furthermore, movements stimulated by substance P [29], NMDA receptor antagonists [30], and D1 and D2 agonists [31] are attenuated following systemic administration of inhibitors of NO–GC signaling. Systemic administration of NOS inhibitors also potentiates catalepsy observed following treatment with DA D2 receptor antagonists [25].

In the presence of normal DA innervation, striatal NO signaling is thought to promote locomotor activity via facilitation of DAergic transmission [8].

A complimentary role of DA and NO in regulating striatal output is also indicated by studies showing that increased striatal levels of cAMP and cGMP observed in phosphodiesterase 1B knock-out mice results in exaggerated locomotor hyperactivity in response to DA agonists [32]. Because cyclic nucleotide phosphodiesterases can metabolize both cAMP and cGMP and restrict the diffusion and action of these second messengers [33, 34], these proteins are uniquely positioned to coordinate the integration of glutamatergic and dopaminergic transmission in striatal MSNs. Taken together, these findings suggest that both DA-stimulated cAMP and NO-stimulated cGMP production play a critical role in the generation of locomotor behavior.

Impact of Partial Striatal DA Depletions on Motor Behavior

Substantial neurodegeneration of nigrostriatal DA neurons is detected in PD and leads to postsynaptic changes in the neurochemical environment of striatal neurons [11]. To investigate what occurs prior to significant loss of striatal DA

and the onset of obvious motor symptoms, we studied rats which were lesioned unilaterally via the injection of a moderate dose of 6-OHDA into the substantia nigra [35]. Once in a week for 4 weeks, rats were evaluated by video taping their forelimb use (Fig. 11.1 A). Rats preferred to use the forepaw ipsilateral to the DA loss (normal paw), compared to the forepaw contralateral to the DA loss (PD paw), or use both forepaws (Fig. 11.1A). Our previous studies have shown that forepaw use patterns are stabilized at 4 weeks after 6-OHDA lesions and continue to be maintained out to 14 weeks, which is the longest time point we have examined [35]. The ratio of limb use of the PD forepaw in comparison to the normal forepaw establishes the DA content in the DA-depleted striatum [36]. We also have related the asymmetrical limb use to tyrosine hydroxylase immunofluorescence (Fig. 11.1B) that detects residual nigrostriatal DA terminals. This approach is highly reliable [35] and allows for the further examination of components of the striatal cGMP signaling cascade using the same brains.

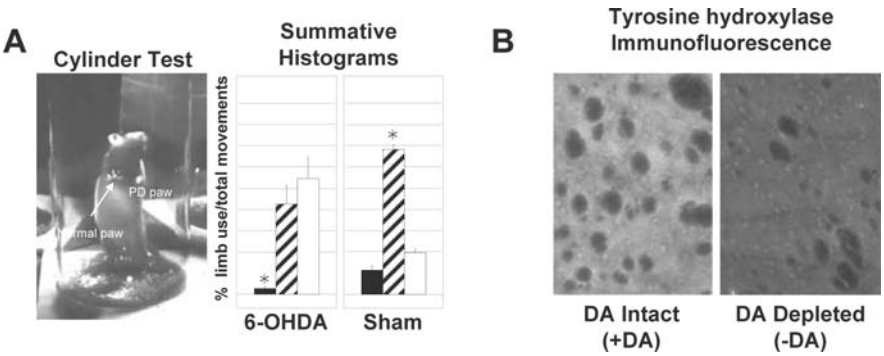

Fig. 11.1 Behavioral evaluation in PD rat model. (**A**) The percentage of total wall touches in a Plexiglas cylinder made with the PD paw, contralateral to the 6-OHDA infusion was diminished significantly in DA-lesioned rats ($n = 20$, *black bar graph* $^*p < 0.05$). The PD paw was held clenched and near the torso, but was not paralyzed and could be used for locomotion and grooming. Co-use of the forepaws (*hatched bar graph*) and touches made with the normal paw ipsilateral to the infusion (*white bar graph*) were compared. The normal paw is touching the wall and the fingers are clearly visible supporting the torso weight as the rat explores vertically in the cylinder. No significant difference in normal paw use in 6-OHDA rats occurred compared to co-use. Summative histograms averaged the total percentage of use of individual or co-use of the paws per total movements (20 maximum movements per session on *X* axis, see Ariano et al. [35]). Paw use patterns in 6-OHDA lesions were contrasted to behavior in sham rats ($n = 4$) which had no preference for use of either paw individually. A significant preference for co-use of the forepaws by the sham rats was present ($^*p < 0.05$). Data were compared by one-way ANOVA followed by Tukey's test post hoc. (**B**) Tyrosine hydroxylase immunofluorescent staining detected the residual nigrostriatal DA terminals in the striatal neuropil. Tyrosine hydroxylase staining was uniform in the neuropil of the dorsal striatum, and not seen in the fiber bundles of the internal capsule, visible as *dark circles* in the DA intact side. Tyrosine hydroxylase staining levels were reduced (59%) in the DA-depleted side compared to the DA intact side (regional luminosities, 34/58, –DA/ + DA)

Impact of DA Depletion on NO-GC Signaling

Several studies have demonstrated that striatal NOS activity is depressed in DA-depleted animals [37–40], but see also work by Chalimoniuk and Langfort [41]. These studies support the outcomes from post-mortem studies of brains from patients with PD showing that striatal nNOS cell numbers and mRNA are reduced significantly in this disease [42, 43]. To characterize the potential impact of partial striatal DA depletions on NOS activity evoked by glutamatergic afferents, the ipsilateral frontal cortex was activated using electrical stimuli delivered for 100 s as trains of high-frequency stimulation (30 Hz, 800 ms train duration, 2 s ITI, see [44]). The duration of the stimulation period (100 s) and the stimulation pattern (30 Hz) were designed to approximate the natural phasic/burst firing activity of cortical pyramidal neurons observed in awake animals [45]. Train stimulation significantly increased NO efflux to a similar degree in the ipsilateral (–DA) and contralateral (+DA) striatum (Fig. 11.2A,B). The increase in NO oxidation signal evoked by train stimulation of the frontal cortex was found to be transient in both the intact and the DA-depleted striata and not significantly different when repeated in the same animal ~20 min after the first or second stimulation trial. These data suggest that in partially lesioned rats, DA depletion does not impact cortically evoked NO efflux.

In contrast to observations of NOS activity, striatal GC mRNA and protein are upregulated in DA-depleted mice [41, 46]. However, studies measuring striatal cGMP levels in DA-depleted animals have reported conflicting results [38, 40, 41, 46]. A potential explanation for these conflicting findings may be that the expression and activity of phosphodiesterases are also upregulated in the DA-depleted striatum [40]. Thus, cGMP synthesis and metabolism may be elevated in MSNs, leading to abnormal intracellular cGMP transients. These studies indicate that complex modifications of

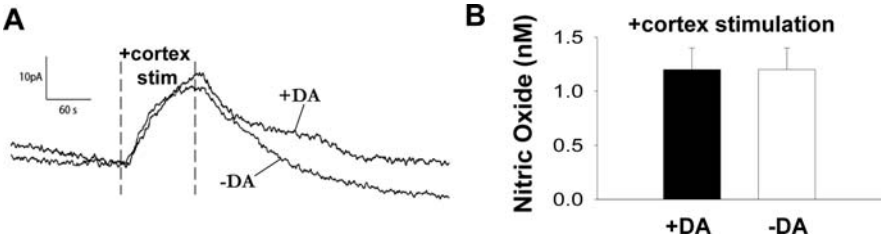

Fig. 11.2 Partial striatal DA depletion does not affect cortically evoked NO efflux in vivo. (**A**) Representative recordings showing the effects of train stimulation (100 s, 30 Hz, 750 μA, 0.5 ms) of the cortex on NO efflux recorded in the contralateral (+DA) or ipsilateral striatum (–DA) of partial 6-OHDA-lesioned rats. (**B**) The mean ± S.E.M. striatal NO efflux evoked during cortical stimulation was not significantly altered in the DA-depleted striatum ($p>0.05$, t-test, $n=16$–28 rats)

GC-cGMP signaling occur following striatal DA depletions. It is also possible that partial striatal DA depletion may have differential effects in striatopallidal versus striatonigral MSNs.

Thus, it is currently unclear how DA depletion will affect NO-cGMP signaling in different subpopulations of striatal MSNs. It has been known for decades that striatal MSNs possess all of the biochemical components of the cGMP signaling cascade [47, 48]. It had not been determined whether the second messenger was distributed preferentially in MSN subpopulations or whether this staining pattern of cGMP and PKG could be modified by manipulating striatal DA levels. To study this further, we employed immunofluorescent staining for cGMP, PKG and DARPP-32 in neuropeptide-identified MSN pathways. Data shown in Fig. 11.3 are representative findings from 10 different 6-OHDA-treated rat striata evaluated for cGMP and PKG. All of the

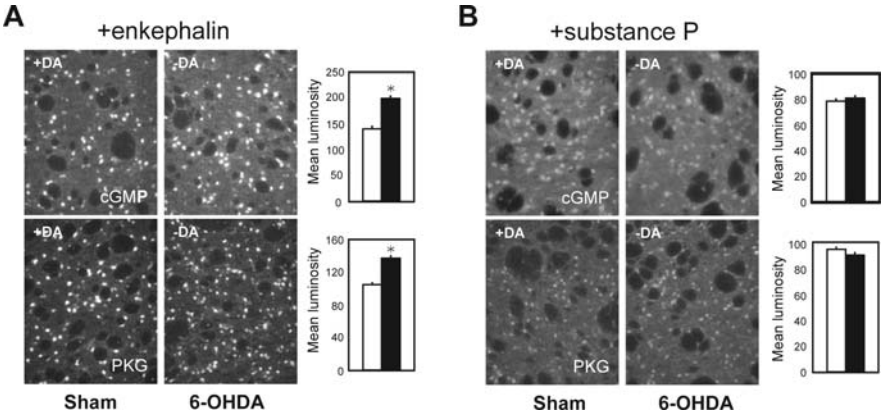

Fig. 11.3 Striatopallidal and striatonigral MSN staining for cGMP and PKG. (**A**) Upper images detected cGMP staining instriatal cryostat sections (10 μm) from the DA intact (+DA) or partially depleted (–DA) striatum in a representative rat which showed limb-use asymmetry and loss of TH staining (–35%; –DA/+DA). Enkephalin-labeled MSNs of the indirect striatopallidal system co-stained with cGMP. Somata luminosity averages were graphed for +DA, 141.3 ± 4.6 (*white bar* in histogram) compared to –DA, 200.4 ± 5 (*black bar* in histogram) and were statistically different (*$p<0.001$). DA loss induced 42% elevation in cGMP signals in striatopallidal MSNs. *Bottom images* show PKG staining in serial sections to the cGMP experiment. Striatopallidal MSNs were identified by enkephalin staining. Somata luminosity averages are graphed to the right for the +DA striatum, 105.2 ± 2.7 (*white bar* in histogram) contrasted to –DA side, 137.7 ± 2.3 (*black bar* in histogram) and were statistically different (*$p<0.001$). DA loss induced 31% elevation in PKG staining levels in striatopallidal MSNs. Similar increases in DARPP-32^{thre34} staining were observed in striatopallidal MSNs (data not shown). (**B**) *Upper images* illustrate cGMP staining as described above for striatopallidal MSNs. Substance P-labeled MSNs of the direct striatonigral system were co-stained for cGMP. The cellular luminosity averages were graphed for +DA, 81.3 ± 1.7 (*white bar* in histogram) compared to –DA, 78.9 ± 1.7 (*black bar* in histogram) and were not statistically different ($p = 0.3$)

6-OHDA-treated rats analyzed displayed limb-use asymmetries and loss of tyrosine hydroxylase staining, ipsilateral to the neurotoxin infusion site; averaged 50% luminosity decrease (range 30–67%). These data clearly distinguish elevated staining in the indirect striatopallidal MSNs versus the direct striatonigral MSNs. Thus, partial disruption of the DA nigrostriatal pathway produces enhancements in the cGMP signaling cascade and these changes are selectively detected within the indirect, striatopallidal MSNs. Indeed, a better understanding of the complexities of cGMP signaling in identified MSNs in the DA-depleted striatum may lead to the development of new strategies for restoring motor function in PD.

Impact of DA Depletion on Striatal MSN Activity and Striatal Output

Loss of striatal DA modulation triggers a variety of neurochemical, metabolic, anatomical and electrophysiological alterations that lead to persistent changes in the activity of striatal MSNs and striatal output [11]. Elevations in D2 receptor protein and mRNA occur along with increases in enkephalin expression in MSNs of the indirect pathway [16]. MSNs of the direct pathway exhibit D1 receptor supersensitivity together with a decrease in overall D1 receptor expression [16]. Corticostriatal terminals also express D2 receptors, [49] which become hypersensitive following DA depletion [50–52]. The loss of the dampening effect of D2 heteroreceptors on corticostriatal terminal excitability results in enhanced glutamatergic transmission and altered NMDA receptor function [31, 53–55]. Complex alterations in dendritic morphology also have been reported in both DA-depleted rats and patients with PD [56–60], which may occur selectively on striatopallidal MSNs (see [61] for review). Most pathophysiological models and metabolic studies of PD predict that the net effect of these alterations is an imbalance in striatal output in which the indirect pathway becomes functionally hyperactive and the direct pathway is slightly hypoactive [62, 63]. In support of this, striatopallidal neurons recorded in DA-depleted mice exhibit a selective loss of endocannabinoid-dependent LTD [64]. Additionally, striatopallidal neurons in DA-depleted animals become more responsive to corticostriatal inputs and as a result, exhibit bursts of spike activity which correlates with cortical oscillations [20, 65, 66]. Thus, loss of D2 receptor-mediated inhibition of striatopallidal neurons and their corticostriatal inputs results in the unfiltered spreading of cortical rhythms to the globus pallidus and other nuclei of the basal ganglia and some of the modifications in neuron activity that may underlie the pathophysiology of PD [67]. Unfortunately, the mechanisms underlying this dysregulation of corticostriatal information processing are not well understood.

To examine the impact of partial striatal DA depletion on cortically evoked activity, we performed extracellular recordings in vivo in the striatum of sham-operated or 6-OHDA-lesioned rats. Electrodes were advanced slowly through the striatum while low-frequency electrical stimuli were applied to the frontal cortex (Fig. 11.4A). We observed that striatal neurons recorded in DA-depleted rats were significantly more likely to spike in

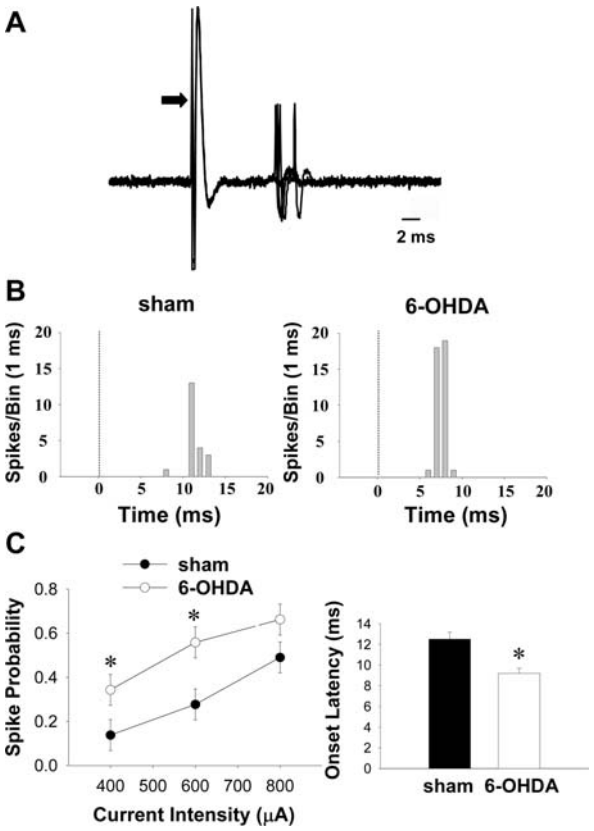

Fig. 11.4 Partial striatal DA depletion increases cortically evoked activity in vivo. (**A**) Representative examples of three overlayed spikes evoked by stimulation of cortex. *Arrow* indicates stimulus artifact. (**B**) Examples of peri-stimulus time histograms (50 trials) depicting the net response of neurons recorded in sham-operated and 6-OHDA-lesioned rats. The *dotted line* at time 0 indicates delivery of the stimulation pulse. (**C**) *Left* The mean ± S.E.M. spike probability of striatal neurons in response to frontal cortex stimulation (at 400 and 600 μA) was elevated in 6-OHDA-lesioned rats as compared to sham-operated rats ($n = 23–25$ cells/3–4 rats per group;$p<0.001$, two-way ANOVA, Tukey's post hoc test $*p<0.05$). *Right*: The mean ± S.E.M. onset latency of spikes evoked by frontal cortex stimulation (800 μA) was decreased significantly in 6-OHDA-lesioned rats as compared to sham-operated rats ($n = 23–25$ cells/3–4 rats per group; $p<0.001$, *t*-test $*p<0.001$)

response to cortical stimulation delivered at the lower current intensities (400–600 μA) compared to sham-operated controls (Fig. 11.4 B,C). Additionally, the onset latency of evoked spikes was significantly shorter in cells recorded from 6-OHDA-lesioned animals compared to sham-operated controls (Fig. 11.4 C). No significant differences in the proportion of spontaneously firing striatal cells were observed between the DA-depleted (12 cells active, 13 cells silent) and the sham-operated groups (13 cells active, 10 cells silent; $p>0.05$, Fisher exact test). Also, no significant differences in the firing rate of spontaneously active striatal cells was observed between the DA-depleted ($0.23 \pm .13$ Hz) and the sham-operated control groups ($0.10 \pm .03$ Hz, $p>0.05$, t-test). These observations indicate that striatal DA depletions increase the responsiveness of MSNs to corticostriatal glutamatergic transmission.

Given the above, it is likely that DA depletion disrupts the modulation of glutamate afferents to striatal MSNs and increases responsiveness to cortical stimulation. The cellular mechanism underlying the elevated glutamate response is unknown. We believe that GC-cGMP signaling has a crucial role in mediating this neuroadaptation and have begun to test this by assessing cGMP signaling in 6-OHDA-lesioned rats. In vivo intracellular recordings performed in intact animals have shown that striatal MSNs exhibit characteristic shifts in membrane potential consisting of a depolarized plateau potential or (up state) and a hyperpolarized resting membrane potential termed the (down state) (see [14] for review). The transition from the down state to the up state is primarily driven by glutamatergic afferents [68]. Indeed, measurements of the amplitude, duration and frequency of spontaneous up states provide important information regarding the responsiveness of MSNs to cortical input. Examples of recordings of spontaneous two-state membrane activity using intracellular recording techniques are shown in Fig. 11.5. MSNs were recorded in sham-operated controls or 6-OHDA-lesioned rats for at least 5 min and time-matched comparisons of basal activity were made. Striatal MSNs were recorded using electrodes filled with control electrolyte (3 M potassium acetate and 0.5 % DMSO) or electrolyte containing the GC inhibitor ODQ (100 μMDA depletion did not affect the up state amplitude or frequency of up events). Consistent with previous studies [65], the duration of up events was enhanced significantly in 6-OHDA-treated rats (Fig. 11.5 B). As observed in naive animals [21], ODQ application significantly reduced the amplitude of spontaneous up states in 6-OHDA-lesioned rats (Fig. 11.5 C). Interestingly, the increase in up state duration observed in 6-OHDA-lesioned rats was reversed by intracellular application of ODQ (Fig. 11.5 C). These findings suggest that cGMP signaling contributes in an important manner to the over-activity observed in MSNs in the DA-depleted striatum.

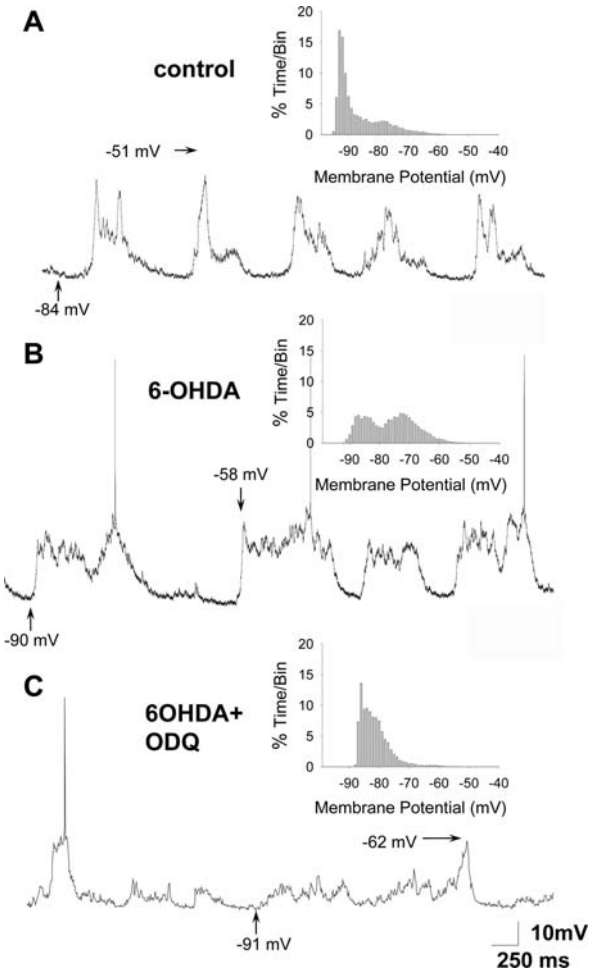

Fig. 11.5 DA depletion increases the duration of spontaneous up events: reversal by intracellular application of the GC inhibitor ODQ. Representative traces of striatal MSNs recorded in a sham-operated (**A**), 6-OHDA-lesioned (**B**) and 6-OHDA-lesioned rats following intracellular application of ODQ (**C**). All MSNs exhibited two-state membrane activity (inset: 30 s; recordings sampled at 10 kHz). MSNs recorded in rats receiving a partial DA depletion exhibited significantly longer up-state durations than their counterparts recorded in control rats or in 6-OHDA rats using electrodes containing the GC inhibitor ODQ (100 μM). *Arrows* indicate the membrane potential at its maximal depolarized and hyperpolarized levels. As previously shown in control rats (West and Grace [21]), intracellular application of ODQ decreased the amplitude of up events in 6-OHDA-lesioned animals. Moreover, intracellular application of ODQ normalized the duration without affecting the frequency of up states observed in cells recorded from 6-OHDA-lesioned rats

Conclusions

While the cellular mechanisms underlying 6-OHDA-induced neuroplasticity are unknown at present, our electrophysiological data strongly suggest that GC-cGMP signaling may contribute to some of these effects. These findings, together with observations of heightened staining of cGMP, PKG and downstream targets of this cascade (e.g., DARPP-32) within the cell bodies of striatopallidal MSNs in the DA-depleted striatum indicate that the NO–GC signaling cascade is enhanced functionally in 6-OHDA-lesioned rats. The activation of striatal nNOS by glutamatergic afferents was not altered in the DA-depleted striatum. However, increased postsynaptic responsiveness to NO and heightened cGMP production is likely to contribute to the hyperactivity of the indirect MSN circuit in the basal ganglia. Furthermore, the current observations are interesting in light of our previous in vivo electrophysiological studies showing that cGMP plays a vital role in amplifying glutamatergic transmission and facilitating membrane oscillations [21, 44]. Thus, augmentation of corticostriatal transmission by NO–GC–cGMP signaling may contribute to the enduring changes in oscillatory activity and membrane excitability of MSNs observed in the DA-depleted striatum particularly in striatopallidal cells. Moreover, selective targeting of NO effector pathways in striatal MSNs may be a promising approach for treating neurological disorders such as PD which may involve over-active excitatory transmission.

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Chapter 12
Dopamine–Endocannabinoid Interactions in Parkinson's Disease

Sarah E. McCallum and Joseph F. Cheer

Introduction

Cannabinoids have been used for medicinal purposes for millennia, functioning as analgesics and anxiolytics but also treating disorders ranging from constipation and digestive ailments to arthritis and malaria. For this reason, it is quite understandable that since their discovery 15 years ago, researchers have explored the uses of endogenous cannabinoids (eCBs) in the same manner. eCBs, lipophilic signaling molecules, affect the signaling of many neurotransmitter systems, and their modulation of dopamine neurotransmission, in particular, has relevance for treating conditions ranging from addiction to movement disorders such as Huntington's and Parkinson's diseases. In the following chapter, we will characterize the relationship between eCBs and dopamine in the nigrostriatal pathway, detailing both dopaminergic modulation of eCBs and the effect of eCBs and CB_1 receptor ligands on dopaminergic transmission. The efficacy of CB_1 receptor modulating drugs for the treatment of Parkinson's disease has been examined in several animal models of the disease. However, to date, it remains undetermined whether eCBs serve as neuroprotective agents or rather, potentiate dopaminergic loss associated with Parkinson's disease (PD). We will discuss interactions between eCBs and dopamine neurons, and propose, based on compelling evidence of eCB modulation of dopaminergic transmission, that CB_1 receptor antagonists are better suited for the treatment of PD.

Overview of the Endogenous Cannabinoid Signaling System

Understanding the effects of cannabis on the CNS has been greatly enhanced by the isolation, cloning and characterization of a receptor that selectively binds cannabinoid compounds. This binding site exhibits stereoselectivity and high

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affinity for agonist ligands and appears to satisfy the criteria for G-protein linkage to other cellular processes [1]. Based on this assumption, an oligonucleotide probe based on the G-protein-coupled receptor for substance K was synthesized and used to isolate, from a rat library, a clone encoding for an "orphan receptor" (a receptor with a unique sequence and no known ligand). The receptor was then expressed by transfection of its complementary DNA (cDNA) in host cells and several ligands were tested for their ability to selectively bind to membranes prepared from these cells. Only the cannabinoids were found to bind to these preparations and to inhibit adenylate cyclase-catalyzed cAMP formation. The central cannabinoid receptor belongs to the seven transmembrane domain family with a glycosylated extracellular N-terminal domain and an intra-cellular C-terminal domain involved (together with the intracellular loops) in the interaction with the G-protein responsible for the transmembrane signal transduction of the receptor-mediated signal.

The knowledge of the genomic and amino acid sequence of the central cannabinoid receptor had immediate consequences. It was now possible to synthesize oligonucleotide probes for the analysis and quantitation, assisted also by polymerase chain reaction or in situ hybridization technology, of the messenger RNA encoding the receptor in several tissues [2]. To date, two central and peripheral cannabinoid receptors have been identified, CB_1 and CB_2. As CB_2 receptor expression is limited to the periphery, our discussion will focus on CB_1 receptors.

Cannabinoid Receptor Distribution in the Brain

Autoradiographic studies indicate that the highest CB_1 density occurs in the molecular layer of the rat cerebellum, basal ganglia (including the nucleus accumbens), substantia nigra *pars reticulata* and the hippocampal dentate gyrus. Dense binding also occurs in the cerebral cortex and other regions of the hippocampus. Moderate levels of binding are found in the hypothalamus, basal amygdala, central grey, nucleus of the solitary tract and laminae I-III and X of the spinal cord. Sparse binding characterizes the thalamus, most of the brain stem, primary sensory, visceromotor and cranial motor nuclei and the *area postrema* [2]. The overall distribution of CB_1 receptors is a unique heterogeneous pattern that does not resemble other receptor maps in that it possesses high forebrain densities and is very scant in brain stem regions. Furthermore, the distribution of this receptor appears to be relatively conserved across species, including humans, primates and rats [2, 3]. The CB_1 distribution pattern may serve to explain most of the physiological actions of cannabinoids. In particular, CB_1 receptors in the cerebellum and the basal ganglia are likely to be responsible for cannabinoid effects on locomotion (discussed below). Cannabinoid receptor mRNA expression is high in a number of brain areas not enriched in receptor binding, and vice versa. These discrepancies can be

explained by the fact that a large number of cannabinoid receptor molecules reside on axons and at nerve terminals that are distant from the cell bodies, which contain the message for their transcription.

Endogenous Cannabinoids

Devane and collaborators [4] postulated that an endogenous cannabinoid (eCB) ligand was likely to be a highly lipophilic agent, and therefore undertook the isolation of Δ^9-THC-like substances from lipid extracts of porcine brain. An ethanolamide derivative of arachidonic acid was isolated and found to bind to the CB_1 receptor and to inhibit electrically stimulated contractions of smooth muscle much in the same fashion as Δ^9-THC. This compound was named anandamide, from the Sanskrit word "ananda", meaning bliss. During the 4 years following its discovery, anandamide was shown to share with Δ^9-THC and other cannabinoids most of their pharmacological properties in both the CNS and peripheral systems [5]. It inhibits adenylyl cyclase, N-type calcium channels [6] and Shaker-related Kv1.2 K + channels (IC50, 2.7 μM) that are found ubiquitously in the mammalian brain [7]. Hampson and colleagues demonstrated that anandamide can also modulate NMDA receptor activity in addition to its role as a cannabinoid receptor ligand [8]. Anandamide (but not Δ^9-THC) was found to augment NMDA-stimulated currents in Xenopus oocytes expressing cloned NMDA receptors. Furthermore, the effects of cannabinoids on Ca^{2+} currents, which are generally associated with NMDA receptors were also explored [9]. The authors found that maximal inhibition of this conductance was achieved with the nonclassical cannabinoid agonist, CP55,940 and anandamide. N-type (omega-conotoxin-GVIA-sensitive) and P/Q-type (omega-conotoxin-MVIIC-sensitive) channels in cultured rat hippocampal neurons carried a Ba^{2+} current modulated by the cannabinoids. The observed inhibition was reduced by the CB_1 receptor antagonist rimonabant (SR141716A), demonstrating a CB_1 receptor-mediated inhibition of distinct Ca^{2+} channels in central neurons. Anandamide's onset of action was later shown to be more rapid than that of Δ9-THC but it was also more quickly degraded than Δ^9-THC [10].

Besides mimicking Δ^9-THC's basic actions in the "tetrad" of behavioral tests in rodents [5], and on the intra-cellular second messengers cAMP and Ca^{2+} [11], anandamide also appears to inhibit motor activity and turning behavior [12, 13]. The tetrad includes evaluation of spontaneous activity in the "open field", immobility index (catalepsy) in the "ring test", analgesia in the "hot plate" test and changes in rectal temperature. Cross-tolerance to Δ^9-THC for some of these effects, i.e., decrease of motor activity in an open field, catalepsy on a ring, hypothermia, analgesia on a hot plate [14], has also been observed with high doses of anandamide, thus further substantiating that this

endogenous metabolite acts, at least in part, through the same mechanism as Δ^9-THC. Finally, anandamide, like Δ^9-THC, was found to increase rat cerebellum and hippocampus cannabinoid receptors turnover after chronic exposure [12]. Other cannabimimetic polyunsaturated *N*-acyl-ethanolamines, found in pig brain at much higher concentrations than anandamide, have been isolated (e.g., 2-arachidonoyl-glycerol; 2-AG). 2-AG is a monoglyceride that produced the tetrad of cannabinoid behavioral actions and was shown to bind CB_1 receptors transiently expressed in COS-7 cells [15]. 2-AG is present in brain in levels higher than those of anandamide. Two metabolic routes exist for both anandamide and 2-AG [16]. Anandamide (or palmitoyl-ethanolamide) is produced by either a synthase enzyme or *N*-acyl-phosphatidyl-ethanolamine-specific phospholipase D. Following membrane depolarization it is released outside the cell and acts at neighboring cells. In order to be catalyzed by the synthase enzyme, the formation of arachidonic acid has to be preceded by phospholipase A2 activation. Once released by cells, anandamide or palmitoyl-ethanolamide can be taken up by selective carrier mechanisms and degraded by fatty acid amido-hydrolase (FAAH), thereby producing arachidonic acid, ethanolamine and fatty acids. The biosynthesis of 2-AG was shown to be stimulated by ionomycin (as with anandamide) in neuronal cells [17]. In mouse neuroblastoma cells, this effect was Ca^{2+} dependent, accompanied by 1-acyl-2-arachidonoyl-glycerol formation, potentiated by incubation of cells with exogenous phospholipase A2 and not sensitive to an inhibitor of one of the enzymes responsible for 1-acyl-2-arachidonoyl-glycerol formation in cells, i.e., phospholipase C. It has been suggested that 2-AG biosynthesis may follow either phospholipase A2- or phospholipase C-mediated pathways depending on whether the neuron is stimulated with agents that are coupled only to Ca^{2+} influx or also to phospholipase C activation.

Clearance of anandamide and 2-AG from the extracellular space is rapidly accomplished via a high-affinity, selective, saturable, temperature-dependent process, suggesting carrier-mediated transport. This transport process is independent of $Na+$ and cellular energy, suggesting a process of facilitated diffusion. AEA and 2-AG compete for uptake, indicating a common transporter. This same transporter may also facilitate diffusion of newly synthesized eCB to the extracellular space [18–20]. Supporting the existence of an AEA transporter, a specific inhibitor of AEA transport has been recently identified [21]. Once taken up into cells, AEA is degraded by FAAH [22–24], and 2-AG is degraded by monoacylglycerol lipase [25].

Endogenous Cannabinoids: Mode of Action in the CNS

A particularly unconventional, recently discovered mechanism is eCB-mediated plasticity. Here, postsynaptic depolarization (via ionotropic or metabotropic receptors) induces production of a lipid eCB messenger that moves

backward across the synapse, binds to presynaptic CB_1 receptors and suppresses transmitter release either transiently or in a consolidated long-term form. There are many known types of eCB-mediated plasticity, acting only at certain synapses and with a specific set of requirements for induction. Unique forms of eCB-mediated synaptic suppression have been reported at excitatory and inhibitory synapses in various brain structures. Where eCB production is triggered by activation of glutamatergic synaptic inputs, eCBs can homosynaptically suppress these same glutamatergic inputs, forming an autoregulatory loop or heterosynaptically suppress nearby GABAergic inputs [26].

A retrograde messenger had long been hypothesized to explain how postsynaptic induction could result in presynaptic expression. In 2001, Wilson & Nicoll [27] published the first report that an eCB is the retrograde messenger that mediates hippocampal DSI. At the same time, working in different systems several other investigators showed clear data implicating eCB signaling in short-term forms of synaptic plasticity [28–32]. These first reports convincingly established that eCBs are important mediators of short-term plasticity. More recent evidence shows that postsynaptic eCB production that is sufficient to cause transient, retrograde, synaptic suppression can be induced either by direct depolarization or by certain patterns of synaptic stimulation [33–35]. Examples of eCB-mediated long-term plasticity at excitatory synapses have been reported in a number of brain structures including striatal [36, 37] synapses.

As in eCB-mediated short-term plasticity, all known cases of eCB-mediated long-term plasticity take the form of depression (eCB-LTD). Certainly, the discovery of eCB-LTD has expanded our view on the role of eCB signaling in the brain, as it becomes apparent that eCBs can have a long-term impact in brain function. Induction of eCB-LTD requires the activation of presynaptic CB_1 receptors, and in most cases, it is expressed presynaptically as a long-lasting reduction of transmitter release. Once established, maintenance of eCB-LTD does not require continued activation of CB_1 receptors. The eCB is released by the postsynaptic cell in response to triggers similar to those that induce eCB-STD, usually a Ca^{2+} rise and/or activation of group I mGluRs (mGluR-I) on the postsynaptic cell. Whereas several common forms of long-term synaptic plasticity critically depend on NMDA receptor-mediated Ca^{2+} influx for induction [38], all known forms of eCB-LTD are independent of postsynaptic NMDA receptors.

Dopamine, Cannabinoids and Movement

The Role of the Basal Ganglia in Movement

The basal ganglia are responsible for the initiation, coordination and execution of movement. This structure is comprised of five major nuclei: caudate,

putamen, substantia nigra, globus pallidus and subthalamic nucleus. The nigrostriatal pathway, the basal ganglia circuit consisting of cell bodies in the substantia nigra compacta that terminate in the dorsal striatum (caudate and putamen) is one of the major dopaminergic pathways in mammalian brain. This dopaminergic pathway is a critical one, primarily since its degeneration is the hallmark of Parkinson's disease, in which dopaminergic neurons in the substantia nigra are lost, and subsequently too, striatal dopamine. The major symptoms of Parkinson's disease such as bradykinesia, rest tremors and rigidity are indicative of dysfunctional motor coordination.

Although it is known that striatal dopamine plays a critical role in modulating the function of glutamatergic medium spiny neurons, which comprise 90–95% of all striatal neurons [39], the precise role of nigrostriatal dopamine as it pertains to the regulation of movement remains to be determined. Evidence from electrophysiological studies in freely moving animals has failed to directly link changes in dopaminergic neuronal activity with locomotor activity per se. Rather, changes in firing rate associated with the presentation of "salient" stimuli have been observed, linking nigrostriatal dopamine with the intention rather than the execution of movement [40–42].

The classically accepted view of basal ganglia function was originally proposed nearly two decades ago [43, 44]. In this model, two pathways exist: the indirect, or striatopallidal, pathway leading from the striatum and projecting to the globus pallidus external (GPe) and subthalamic nucleus (STN) loop and the direct pathway, or striatonigral, which projects to the substantia nigra pars reticulata (SNr)/globus pallidus pars interna (GPi). The two pathways work in tandem to inhibit (indirect) and facilitate (direct) movement via the inhibition and disinhibition of the thalamus. Although co-localization of D1 and D2 dopamine receptors has been reported to occur in a small percentage of neurons [45]; a simple view assumes that primarily D2 receptors comprise the indirect pathway and D1 receptors are localized within the direct pathway. Parkinson's disease is believed to represent a dysfunction of this coordinated function. One hypothesis is that dopamine depletion causes a cascade of downstream events, beginning with the disinhibition of neurons along the indirect pathway (D2 receptor-expressing). This, in turn, leads to the disinhibition of the STN and concomitant increased inhibition of neurons in the GPe. These changes are followed by overexcitation of neurons in the GPi/SNr (direct pathway), which results in the suppression of areas of motor control in the cortex, thalamus and brain stem. This model is supported by evidence from experimental studies as well as from the clinic. For instance, lesions of the STN in MPTP-treated monkeys improve parkinsonian symptoms [46, 47]. Furthermore, evidence from clinical studies suggests that deep brain stimulation of the STN improves parkinsonian symptoms [48].

In animal models, a better understanding of the role of dopamine receptors in movement, specifically turning behavior, can be achieved with a unilateral dopamine depletion, as occurs following local administration of the selective dopaminergic toxin, 6-hydroxydopamine. Dopaminergic denervation results in

an upregulation of both D1 and D2 dopamine receptors. Although this method has been instrumental in elucidating the individual roles of D1 and D2 receptors in movement, it should be noted that one complication arising from unilateral dopamine depletion is the potential for undoing existing D1/D2 synergism, which may provide misleading information about the role of each of these receptors in movement [49].

Dopamine D1 Receptor-Mediated Changes in Motor Activity

Within the basal ganglia, the striatum performs the function of processing center. Here, stimulation of dopamine D1 receptors on medium spiny neurons that project to the SNr and SNc increases locomotor activity in rats. This is in agreement with the functional model discussed above, as one would expect that direct pathway stimulation (via dopamine D1 receptor agonist administration) would inhibit SNr/GPi output, disinhibiting the inhibitory action of the thalamus on movement. While systemic injections of dopamine D1 receptor agonists typically produce increased locomotor activity in rats [50, 51], it is difficult to fully attribute increases in locomotor activation to activation of striatal dopamine D1 receptors following systemic administration.

Intrastriatal injections of the dopamine D1 receptor agonists in intact animals have yielded mixed results, but taken together, the findings suggest increases in motor activity can be attributed to D1 receptor activation. For instance, local injection of the D1 agonist SKF82958 increases overall locomotor activity as well as an increased observation of stereotypy both in control animals and rats lesioned with 6-OHDA [52]. In another study, intrastriatal injection of the D1 agonist SKF 81297 affected neither rotational behavior nor locomotor activity, while motor activity was decreased with a D2 agonist [53]. When a D1 agonist was locally administered to rats in conjunction with a D2 antagonist, in order to isolate D1 effects, a small D1 receptor-mediated increase in oral movements was observed [49]. Dopamine D1 receptor-mediated increases in activity are supported by evidence of decreased spontaneous locomotor activity in mice lacking D1 receptors [54].

Dopamine D2 Receptor-Mediated Changes in Motor Activity

In keeping with the historical model of basal ganglia function, it would be expected that intrastriatal delivery of a D2 dopamine receptor agonist would inhibit movement. Indeed, when administered alone in intact animals, intrastriatal injections of quinpirole, a D2 agonist, reduce locomotor activity [53]. Isolating D2 receptors by administering quinpirole concurrently with a D1 antagonist, SCH 23390, administered bilaterally into the striatum, resulted in a suppression of motor activity in rats [49]. In a recent paper by Welter et al.

[55], D1 receptors were isolated using a D2 receptor knockout mouse strategy. These mice exhibited abnormal responses to cocaine, illustrating the inhibitory role of D2 receptors on dopamine signaling. Finally, a systemic injection of a D2, but not a D1 agonist, in dopamine-depleted rats decreased locomotor activity in rats [56]. Taken together, these studies suggest that inhibition is the hallmark effect of D2 receptor activation on locomotion.

Cannabinoid Receptor Activation and Movement

It is well recognized that cannabinoids affect psychomotor performance. Therefore it is not surprising that the ability to perform certain tasks can be compromised in some individuals following marijuana smoking. There is little dispute that high doses of marijuana can disrupt performance when the task is difficult. Cannabinoid-induced impairment of flying and driving has been documented. As might be expected, the effects of marijuana on performance become more variable as the complexity of the task is simplified and the dose of marijuana is reduced. In their review, Chait and Pierri [57] concluded that marijuana, at doses that produce moderate levels of intoxication, can affect a wide range of learned and unlearned behaviors, including simple motor tasks and more complex psychomotor and cognitive tasks.

The high density of CB_1 receptors in the basal ganglia and cerebellum is highly indicative of their role in extra-pyramidal motor function. Cannabinoids are also known to affect extra-pyramidal motor function. Animal studies of the effects of cannabinoids on motor activity have been summarized elsewhere [58]. In general, as would be expected based on its effects in humans, systemic administration of phytocannabinoids such as Δ^9-THC induces locomotor depression and catalepsy in rodents [59, 60], effects that are blocked by rimonabant [61]. Synthetic CB_1 receptor agonists such as WIN 55,212-2 and CPP 55,940 also elicit catalepsy in rats [62].

As for the effects of the endogenous cannabinoids on motor behavior, several investigators have found that administration of anandamide produces the expected inhibition of motor behavior in rats and mice [63–67]. In work by Romero and collaborators [12], the administration of anandamide decreased the ambulation and the frequency of stereotypic movements (in particular, the number of rears) and increased the time spent by the rats in inactivity. These effects were evident at 10 and 30 min after the administration of the cannabinoid agonist, but mostly disappeared at 60 min. Interestingly, motor inhibition was observed again around 2 or 3 h after the administration of anandamide. This was a small but persistent effect (decreased ambulation followed by increased inactivity), because it was observed until at least 6 h after anandamide administration [12]. R-methanandamide inhibits motor behavior in a manner (its effects were persistent) that resembles the effects of Δ^9-THC rather than the effects of anandamide (its effects were of rapid onset but shorter duration) [68].

Stein et al. [67] noted locomotor depressant, antinociceptive and hypothermic effects in rats after acute, intravenous administration of anandamide. Similar effects of eCBs on locomotor activity have been observed in several mouse strains. Chakrabarti et al. [63] reported effects ranging from locomotor depression to catalepsy after subchronic administration of anandamide to C57BL/6, ICR and to a lesser extent, DBA/2 mice. Finally, the anandamide transport inhibitor, AM404, produces hypokinesia in rats [69]. Taken together, these findings reinforce the notion of a cannabinoid receptor-mediated control of nigrostriatal function.

Based on the locomotor depressing effects of exogenous and endogenous cannabinoids, it might be expected that CB_1 receptor antagonists such as rimonabant would have an opposite effect (i.e., hyperlocomotion) in laboratory animals. Indeed, hyperlocomotion in laboratory animals has been reported [70], but has not been consistently observed. Rather, rimonabant blocks hyperactivity caused by psychomotor stimulants such as cocaine [71, 72] while having no effects per se on locomotor responses in animals.

Convergence of Dopamine and Cannabinoid Receptor Activation: Implications for Motor Control

One proposed role of endocannabinoids in the basal ganglia is that of a "brake" on glutamatergic and dopaminergic activity in the striatum. In this way, rimonabant increases behavioral activation (stereotypies) induced by combined administration of D1 and D2 receptor agonists [73]. Conversely, the potent CB_1 receptor agonist CP 55,940 enhances catalepsy elicited by D1 or D2 receptor antagonists [74]. However, rotational studies in rats receiving local injections of cannabinoid receptor agonists and antagonists into the basal ganglia suggest that dopamine–endogenous cannabinoid interactions are significantly more complex. For example, administration of CP 55,940 induces contralateral turning when microinjected unilaterally into the substantia nigra pars reticulata [75] and striatum [76]. In another study, local injection of D2 (spiroperidol) or D1 ((+)-SCH 23390) antagonists, or prior 6-OHDA striatal lesions blocked WIN 55212-2- and CP55,940-induced turning, thus suggesting the involvement of dopamine release in cannabinoid-induced turning [13].

Using the unilateral, 6-OHDA-lesioned rat as a model, El Banoua et al. [77] studied the interactions between rimonabant and the D1 and D2 receptor agonists, SKF-38393 and quinpirole, on amphetamine-induced ipsilateral rotations. Interestingly, intrastriatal injections of the SKF-38393 administered in conjunction with rimonabant had a greater functional response (i.e., reduced rotations) than either compound alone. In contrast, rimonabant given in conjunction with the D2 receptor agonist quinpirole resulted in a smaller response than either compound administered alone. These findings suggest that CB1 antagonism positively modulates D1-mediated motor function, but negatively

modulates D2-mediated motor function. These results are compatible with work by Anderson and colleagues [62] who demonstrated that systemic injections of CB1 receptor agonists attenuate contralateral rotations induced by D1 but not D2 receptor agonists. This notion is reinforced by the fact that doses of WIN 55,212-2 and CP 55,940 that attenuate D1-mediated rotation do not produce catalepsy in intact rats or in rats with 6-OHDA -induced lesions, further indicating that the reduction in rotation produced by the cannabinoids is not due to a generalized motor impairment. In addition, the effective dose of WIN 55,212-2, but not CP 55,940, produced only a slight increase in ipsilateral rotation when administered alone, making it improbable that this ipsilateral tendency accounts for the reduction in D1-mediated contralateral rotation.

These results suggest a preferential interaction between cannabinoid receptor stimulation and dopamine D1 receptor-mediated behavior. Taken together, these findings reinforce the notion of a cannabinoid receptor-mediated control of nigrostriatal function.

Dopamine and Endogenous Cannabinoid Interactions in Striatum

Cannabinoids Exert Excitatory Control over Dopamine Neurotransmission

Neurons within SNpc provide dopamine innervation to basal ganglia structures which are involved in the control of motor activity. Thus, SNpc neurons may be a target for the cataleptogenic actions of cannabinoids. Indeed, acute Δ^9-THC increases the activity of dopamine neurons in the SNpc in a dose-dependent manner. Moreover, in animals made behaviorally tolerant to Δ^9-THC through a course of chronic cannabinoid exposure, Δ^9-THC's effects on neuronal firing rate and the amount of bursting activity are rapidly desensitized [78]. The latter electrophysiological parameter may be particularly relevant since it has been shown that action potentials generated in a bursting pattern result in greater synaptic levels of transmitter than that which would occur from the same number of action potentials generated singly [79] and these bursts of activity in the SNpc have been linked to the initiation of goal-directed behavior [41]. Importantly, acute cannabinoid treatment elicits dramatic increases in the number of dopamine concentration transients recorded voltammetrically at dopamine terminals that are similarly time-resolved as bursts of activity at the cell bodies [80]. Similarly, eCBs also have the ability to elevate dopamine levels in terminals fields in striatum, presumably via an action on dopaminergic neurons in the ventral midbrain [81]. The mechanism through which exogenous as well as eCBs exert their actions is complex since CB_1 receptors are not present on dopaminergic neuron cell bodies or terminals [82]. Because of their potent presynaptic inhibition of neurotransmitter release, CB_1 receptors are in an

exquisite position to play an integral role in the control of the two major afferent transmitters, glutamate and GABA, that modulate the activity of midbrain dopamine neurons [82]. Therefore, any change in receptor sensitivity chronic cannabinoid treatment could effectively alter the ratio of the excitatory and inhibitory drives governing the rate and patterns of dopamine cell firing. Such differences in afferent tone could also help explain, for example, why the magnitude of Δ^9-THC-induced excitation is greater in the ventral tegmental area compared to the SNpc of naive animals, and possibly how SNpc neurons become less responsive to Δ^9-THC after chronic treatment. Nonetheless, the mechanism for the lack of tolerance in VTA neurons remains elusive. This is an important consideration since chronic treatment with Δ^9-THC reportedly plays a therapeutic role in rats with 6-OHDA lesions (see Table 12.1).

Endogenous Cannabinoid and Dopamine Dynamics in the Hypodopaminergic State

In 6-OHDA-lesioned rats, many studies have described dopaminergic hypersensitivity resulting from dopamine depletion that is accompanied by greatly elevated expression of striatal D2-like receptor levels [83, 84]. These findings have been recently replicated in a series of elegant experiments that involved the use of dopamine-deficient (DD) mice [85]. In addition, dopamine neurons in DD mice are hypersensitive to the dopamine receptor agonist quinpirole, suggesting a profound upregulation of this particular sub-class of receptor in hypodopaminergic states [86]. Importantly, occupation of D2-like receptors

Table 12.1 Effects of CB1 agonists in animal models of Parkinson's disease

Agonist	Specific action	Animal model	Effect	Reference
		6-OHDA rat	Improved symptoms	[109]
Δ^9-THC	Partial agonist	MPTP cynomolgus monkey	Worsened symptoms	[113]
ACEA	CB1 agonist	6-OHDA rat	No change	[103]
HU-210	CB1 agonist	6-OHDA rat	Improved symptoms	[114]
HU-308	CB2 agonist	6-OHDA rat	Improved symptoms	[103]
WIN55,212-2	CB1/CB2 agonist	6-OHDA rat	No change	[103]
AM404	EC uptake inhibitor	6-OHDA rat	Improved symptoms	[101–103]
UCM 707	EC uptake inhibitor	6-OHDA rat	No change	[103, 102]
	Low-affinity	6-OHDA rat	Improved symptoms	[109]
Cannabidiol	CB1/CB2 agonist	6-OHDA rat	No change	[114]

by dopamine elicits the release of anandamide in striatum and possibly in other regions of the CNS that contribute to movement control [87]. Furthermore, this effect on anandamide content is biphasic with low, autoreceptor-preferring doses increasing tissue levels [88]. By engaging CB_1 receptors, anandamide may act in turn to counter dopamine stimulation of motor activity, which is thought to be mediated by postsynaptic D2-like and D1-like receptors.

Potential for Therapeutic Use of Cannabinoid Receptor Modulators for Parkinson's Disease

Role of Endogenous Cannabinoid Signaling in Parkinson's Disease

Pathology in PD has been traditionally attributed to a profound decrease in ambient levels of dopamine at striatal terminals. However, it is still unclear how such a hypodopaminergic state contributes to maladaptive processing within the basal ganglia. One key component of this maladaptive integration may reside in altered endogenous cannabinoid signaling. Recent findings in untreated PD patients have shown abnormally high levels of anandamide in cerebrospinal fluid [89]. Similarly, increased anandamide and 2-AG levels have been reported in rat models of PD [90]. It is well established that tolerance develops rapidly during chronic administration of cannabinoids [91] with receptor desensitization or uncoupling [92] consistently implicated as one of the molecular events underlying the onset of tolerance. Thus, tolerance to repeated CB_1 receptor activation does not involve changes in pharmacokinetics [93]. Chronic administration of Δ^9-THC leads to an uncoupling of CB_1 receptors from G-proteins, as measured by a decrease in WIN 55,212-2-stimulated GTPγS binding [94]. Therefore, the notion that endocannabinoids are hyperactive in the striatum is also entirely consistent with findings from post-mortem human investigations as well as pre-clinical studies showing decreased levels of CB_1 receptor mRNA specifically within the basal ganglia of PD subjects [95, 96]. Indeed, if receptors are uncoupled because of extensive exposure to endogenous ligand binding, as is the case in PD, the biological consequence at the receptor level is a downregulation of expression [96]. These abnormally elevated levels of endogenous cannabinoids, especially of anandamide, may be generated by hypersensitive low-affinity D2 autoreceptor activity [87, 88] in response to hypodopaminergia. It is likely that these compensatory mechanisms occurring in the striatum of PD patients are aimed at normalizing chronic dopamine depletion. However, the specific contribution of such alterations to the symptoms of PD or to the side effects of treatment remains to be determined.

Therapeutic Use of CB1 Receptor Antagonists

With few exceptions, treatment with rimonabant improves parkinsonian symptoms in several animal models of the disease (see Table 12.2). In the 6-OHDA-lesioned rat model of PD, a low dose (0.1 mg/kg) but not higher doses (0.5–1.0 mg/kg) partially prevents hypokinesia associated with bilateral administration of the toxin [97]. Rimonabant is also effective in non-human primates lesioned with the selective dopaminergic toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [98, 99]. Van der Stelt and colleagues administered rimonabant (0.3, 1.0 and 3.0 mg/kg, p.o.) to MPTP-lesioned marmosets and found that the two higher doses effectively increased motor activity in parkinsonian animals without producing dyskinesias [98]. The concept, that CB1 receptor antagonists may be viable therapeutic alternatives for PD, is at odds with a very recent study by Kreitzer and Malenka [100]. In this study that authors show that indirect-pathway eCB-LTD is absent but is rescued by a D2 receptor agonist or by inhibitors of anandamide degradation. Moreover administration of these drugs together in vivo reduced parkinsonian motor deficits, suggesting that eCB-mediated depression of indirect-pathway synapses plays a critical role in the control of movement. The controversy resides in the concept that by interfering with anandamide degradation, its tissue levels are enhanced. However, it has already been discussed (*vide supra*) that a novel hallmark adaptation observed in PD is an overactivity of eCB signaling accompanied by a decrease in CB_1 receptor expression. If levels of eCBs are already enhanced in PD, raising them further by blocking their degradation could effectively *worsen* the symptoms of PD (unless a ceiling effect is achieved in PD in which case the degradation strategy would be devoid of effects). It is unclear why the authors did not pre-treat animals with a CB_1 receptor antagonist to assess the specificity of the behavioral effects they obtained. Another line of experiments that may argue against the validity in the therapeutic efficacy of CB_1 receptor antagonists in PD relates to several studies that have reported ameliorations in PD symptoms

Table 12.2 Effects of CB1 antagonists in animal models of Parkinson's disease

Antagonist	Specific action	Animal model	Effect	Reference
		6-OHDA rat	Improved symptoms	[77, 97, 115]
		MPTP marmoset	Improved symptoms	[98]
SR171416A (rimonabant)	CB1 antagonist	MPTP cynomolgus monkey	No change	[113]
		Reserpinized rat	Improved symptoms	[90]
CE	CB1 antagonist	MPTP rhesus monkey	Improved symptoms*	[99]
AM251	CB1 antagonist	6-OHDA rat	Improved symptoms	[115]

* Improved symptoms of PD when administered in conjunction with L-DOPA

following blockade of the putative eCB transporter [101–103]. The same logic applies here as in the case of the degradation inhibitor. However, these experiments are further convoluted by electrophysiological findings suggesting that these drugs may block *release* [36, 94, 104] as well as uptake of eCBs. In electrophysiological studies, when extracellular levels of retrograde acting endogenous cannabinoids are indirectly detected on a second to minute time-scale, the results are opposite to those of an indirect agonist; in fact, these compounds may initially depress the release of 2-AG and anandamide [105, 106]. If that is the case, then the effects of blockade of the eCB transporter may equate that of treatment with a CB1 receptor antagonist, because these two approaches result in decreased eCB binding.

Therapeutic Use of CB1 Receptor Agonists

Increases in eCBs have been observed in untreated PD as well as in animal models of the disease. As a role for eCBs in movement control has been identified, a logical conclusion is that increases in eCBs accompany PD symptomatology. Therefore, CB_1 receptor antagonists should be effective in reducing motor symptoms in PD. As discussed above, with few exceptions, this is the case. Furthermore, consistent with the locomotor depressant properties of cannabinoids and other CB_1 agonists, it might be concluded that these compounds would not improve, and perhaps may even worsen, PD symptoms. Plant-derived and synthetic cannabinoids have been administered in animal models of PD and show beneficial effects. These studies collectively give the impression of a beneficial role of endocannabinoids in movement disorders. However, positive effects of cannabinoids on PD symptoms may occur in a CB_1 receptor-independent manner. In fact, the antioxidant and anti-inflammatory properties of natural and synthetic cannabinoids have been suggested to either practically or fully contribute to the beneficial effects of these compounds on the symptoms of PD. Antioxidative properties of cannabinoids have been characterized for which no evidence of CB_1 receptor activation exists [107, 108]. Cannabidiol, a marijuana plant derivative with no psychoactive properties was effective in the 6-OHDA rat model of PD but has no significant activity at cannabinoid receptors [109]. This evidence, coupled with the observation that cannabidiol possesses antioxidative properties not attributable to CB_1 receptor activation [110], suggests that cannabinoid-induced improvements in animal models of PD may not be related to activity at CB_1 receptors.

Concluding Remarks

The studies discussed above provide compelling evidence for a therapeutic role of CB_1 antagonists in PD. Antagonist therapy offers a great advantage over treatment with agonists since the nature of the pharmacotherapy involves

displacement of aberrant endogenous tone from CB_1 receptors rather than overactivation. This is particularly relevant for cannabinoid receptors as they are the most abundant G-protein-coupled receptor in the brain and thus the likelihood of nonspecific side effects is reduced by treatment with rimonabant compared to therapy involving the use of agents that raise tissue levels of eCBs or that bind to CB_1 receptors with high affinity.

Nevertheless, there may be a beneficial role for CB_1 agonists in alleviating levodopa-induced dyskinesias (LIDs) in patients with PD. Although the precise role of eCBs in PD has not been fully determined, the finding that nigrostriatal degeneration is associated with increased levels of eCBs can be further extended to one interpretation involving the use of such agonists in cases where L-DOPA treatment induces neuroadaptations that produce decreases in eCB levels [111]. Indeed, treatment with nabilone (Cesamet™, a synthetic cannabinoid) is successful in treating LID's in Phase II clinical trials [112].

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Chapter 13
Glutamate Plasticity in an Animal Model of Parkinson's Disease

Charles K. Meshul

Although it is well established that in Parkinson's disease there is a significant loss of dopamine within nerve terminals in the striatum, the depletion of dopamine most likely influences other neurotransmitter systems. There is growing interest in the interactions between dopamine and glutamate and it may be the lack of dopamine in Parkinson's disease that results in dynamic changes in glutamate within at least the striatum [1, 2]. In the rodent, the sensorimotor cortex provides the primary excitatory, glutamatergic input to the dorsolateral striatum [3, 4], although recent data suggest that glutamate input from many nuclei within the thalamus may also be playing an important role [5–7]. The dopamine terminals originating from the substantia nigra pars compacta (SN-PC) make a symmetrical synaptic contact not only on the dendritic shaft of the medium spiny neuron but also on the neck of the dendritic spine [8, 9]. The asymmetrical synaptic contact on the head of that same spine within the dorsolateral striatum originates from not only the motor cortex but also the thalamus [5, 7] and the nerve terminals contain the neurotransmitter, glutamate [8, 10, 11]. Not only are dopamine and glutamate terminals anatomically located next to each other, but these two neurotransmitters can control their own release and also the release from each other's nerve terminals [12–15]. In addition, a small percentage of the glutamate nerve terminals originating from the cortex contain presynaptic dopamine D-2 receptors [16, 17]. When these dopamine D2 receptors are activated or blocked, we and others have reported that glutamate release decreases or increases, respectively [12, 14, 18]. Therefore, alterations in the level of striatal dopamine can have profound effects on nearby glutamate synapses.

In Parkinson's disease, there is a slow and progressive loss of dopamine input to the striatum, one of the major nuclei of the basal ganglia. Only when there is

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about a 70–80% loss of striatal dopamine are individuals symptomatic for the disease. The symptoms can include akinesia, rigidity, and tremor [19]. The loss of striatal dopamine affects the output neurons from this brain area that are part of the so-called direct and indirect pathways [20]. Ultimately these pathways merge at the level of motor thalamus. The motor thalamus is then the start of another excitatory projection, leading to the cortex and then back to the striatum (thalamo-cortico-striatal pathway). In animal models of nigrostriatal degeneration, administration of AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole-priopionate) or NMDA (*N*-methyl D-aspartate) glutamate receptor antagonists decrease the parkinsonian symptoms associated with the loss of striatal dopamine and the dopamine agonist-induced motor fluctuations [2, 21–28]. In addition, intrastriatal injection of NMDA leads to parkinsonism [29], suggesting that glutamate synapses within the striatum play an important role in the development of the movement disorders associated with Parkinson's disease.

Time-Dependent Changes in Striatal Glutamate Following Loss of Dopamine

We first investigated changes in glutamate within the dopamine deafferented striatum of rats following a unilateral injection of the neurotoxin, 6-hydroxydopamine (6-OHDA), into the medial forebrain bundle (MFB) [30]. We analyzed changes in striatal glutamatergic function both 1 and 3 months following the lesion. Most studies investigating the effects of an MFB lesion using 6-OHDA are relatively short term (i.e., 1 month or less) and of the very few striatal microdialysis studies carried out that measured glutamate, both appeared to be using time periods of no more than 2 months following the lesion. Therefore, it was important to determine if the near total loss of striatal dopamine would have a long-term affect on glutamate in this brain region. The concentrations of tissue dopamine were analyzed both at the 1- and 3-month time periods and were shown to be nearly completely depleted compared to the sham group [30]. This correlated exactly with subsequent studies using optical density to measure the relative density of tyrosine hydroxylase (TH) immunolabeling within the striatum that also showed a nearly complete loss of TH immunolabeled cells within the substantia nigra pars compacta [31].

Electron Microscopy/Immunocytochemistry

To ensure the success of the 6-OHDA lesion, all rats were first tested with a low dose of apomorphine (0.05 mg/kg, i.p.) 3 weeks after the lesion. Only those animals showing robust contralateral turning (> 10 turns/min) were used in the experiments. The density of glutamate within striatal nerve terminals making an asymmetrical synaptic contact was determined. Although it had been widely

accepted that nerve terminals making an asymmetrical synaptic contact are excitatory and most likely contain the neurotransmitter, glutamate**,** it had never been firmly established with ultrastructural immunocytochemical methods that such nerve terminals actually contained glutamate within the striatum. Using an antibody made against glutamate [32, 33] and a secondary antibody tagged with a 10 nm gold particle, we could then identify and quantify the density of such glutamate labeling within identified nerve terminals making an asymmetrical synaptic contact. We were the first to report that following subchronic treatment with the dopamine antagonist, haloperidol, the changes in asymmetrical synaptic contacts were associated with nerve terminals containing glutamate and that there was an increase in the density of such nerve terminal glutamate immuno-gold labeling compared to the control group [11, 34]. However, there is a heterogeneous glutamate input to the striatum. For example, glutamate-containing nerve terminals making an asymmetrical synaptic contact on a dendritic shaft most likely originate from the thalamus [35, 36]. In the rat, we estimated that such thalamostriatal afferents that contact dendritic shafts account for about 10% of all asymmetrical synaptic contacts [30]. We have been made aware only recently that nerve terminals making an axospinous contact could still originate from the thalamus [5–7]. The terminals from the cortex and thalamus can be differentiated by their vesicular glutamate transporters. The input from the cortex contains the messenger RNA and protein for the vesicular glutamate transporter-1 (VGLUT 1), while that from the thalamus contains the vesicular glutamate transporter-2 (VGLUT 2) [6, 37]. The input from the subthalamic nucleus to the striatum in the rat [38] does not appear to make a direct or significant contribution to striatal extracellular glutamate. A lesion of this nucleus plus the nigrostriatal pathway did not result in any additive changes in extracellular striatal glutamate as compared to a 6-OHDA lesion alone [39]. Although the cortical input to the dorsolateral striatum converges from wide areas of the motor/sensory cortex [4], we have never observed a bimodal effect on the density of nerve terminal glutamate immunolabeling after a 6-OHDA lesion. This suggests that regardless of the origin of the input to specifically the dorsolateral striatum, be they from pyramidal tract cortical neurons or the thalamus, it appears that all glutamate synapses onto dendritic spines within the dorsolateral striatum are affected equally by the nigrostriatal lesion. In our studies, we are determining the relative density of glutamate immuno-gold labeling within nerve terminals and not the actual levels of glutamate since it is not known how many gold particles represent the number of glutamate molecules. Figure 13.1 shows a representative example of a glutamate immunolabeled terminal from a control group. It is important to note the larger accumulation of gold particles within the nerve terminal (NT) compared to the postsynaptic dendritic spine (DS). In addition, we find that most of the gold particles are associated with the synaptic vesicle pool [30].

One month following the lesion there was a decrease in the density of nerve terminal glutamate immunolabeling within all asymmetrical synapses

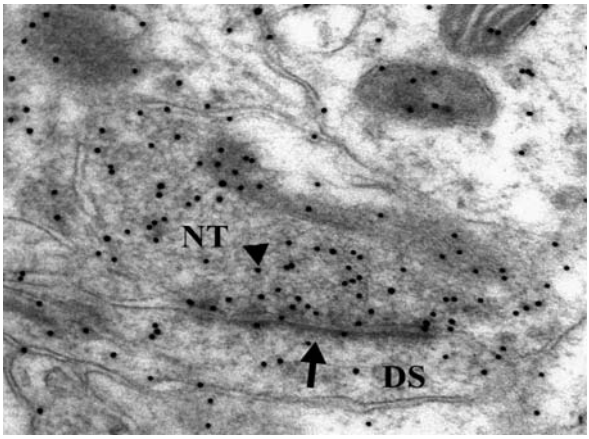

Fig. 13.1 Glutamate immuno-gold-labeled nerve terminal. Electron photomicrograph using the immuno-gold technique to localize an antibody against the neurotransmitter, glutamate, within the dorsolateral striatum. Within the nerve terminal (NT), there is an accumulation of small round synaptic vesicles and 10 nm gold particles indicating the location of the antibody (*arrowhead*). The nerve terminal is making an asymmetrical synaptic contact (*arrow*) with an underlying dendritic spine (*DS*)

(Fig. 13.2). In addition, the change in striatal glutamate at this 1 month time period is most likely not due to sprouting of new glutamate synapses since it has been reported that such a 6-OHDA lesion results in an actual decrease in the density of striatal asymmetrical nerve terminals [40]. Even though striatal dopamine levels do not recover, glutamate synapses continue to show signs of plasticity.

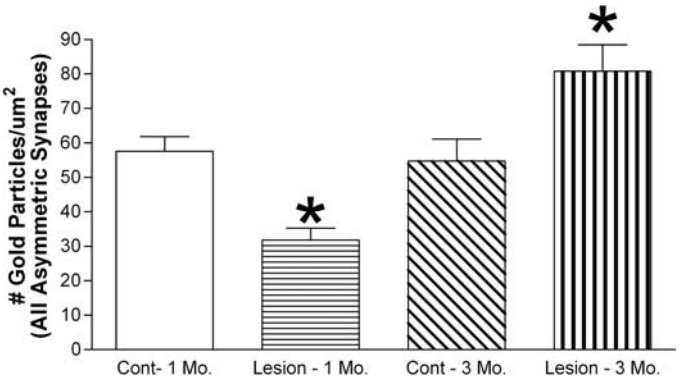

Fig. 13.2 Density of nerve terminal glutamate immuno-gold labeling following loss of striatal dopamine. 1 and 3 months following a nigrostriatal lesion, there is initially a decrease and then an increase, respectively, in the density of glutamate immuno-gold labeling within striatal nerve terminals making an asymmetrical synaptic contact. * – $p < .05$ compared to the respective control group

Within the glutamate-containing nerve terminal, there are potentially several sources of glutamate: cytoplasmic, vesicular, and mitochondrial. At the 1 month time period, the mitochondrial pool reflected changes that were similar to those seen in the vesicular pool [30]. The cytoplasmic pool was very small, accounting for less than 10% of the entire glutamate pool. This pool did not show any significant changes 1 month following the lesion. There were no differences in the density of glutamate within dendritic spines between the groups, demonstrating the specificity of the immunolabeling technique [30].

Of particular interest and surprise was that 3 months following a nigrostriatal lesion, the relative density of nerve terminal glutamate immunolabeling showed an increase compared to the non-lesioned group (Fig. 13.2). This was reflected in both the vesicular and mitochondrial pools of glutamate compared to the cytoplasmic pool, which continued to show no changes [30]. It is not known if the glutamate input from the cortex vs thalamus is differentially affected following a nigrostriatal lesion, but it has been reported that following a toxic regimen of methamphetamine, resulting in long-term loss of striatal dopamine, there is an increase in extracellular glutamate in the striatum and this appears to be associated with an increase in VGLUT-1 vs VGLUT-2 protein levels [41]. This suggests that the increase in striatal glutamate following methamphetamine affects the corticostriatal vs thalamostriatal pathway. However, it has been reported that a lesion of the intralaminar thalamic nuclei (centromedian and parafasicular) blocks the 6-OHDA-induced increase in striatal enkephalin and glutamic acid decarboxylase mRNA and in cytochrome oxidase mRNA in the subthalamic nucleus [42]. This suggests the importance of the thalamostriatal pathway in this ngirostriatal lesion animal model of Parkinson's disease. The increase in nerve terminal glutamate immuno-gold labeling 3 months following the 6-OHDA lesion was similar to that reported by Ingham et al. [40]. But in their study, this increase was found just 1 month after the loss of striatal dopamine. Although dopamine levels were not determined in that study, they used a significantly higher concentration of apomorphine (0.25 vs 0.05 mg/kg) to determine the success of the nigrostriatal lesion. The other major difference between our studies was that Ingham et al. [40] used the contralateral side as the control compared to our study where a sham animal was as part of the control group [40].

This complete reversal in terms of nerve terminal glutamate immunolabeling was not reflected in any changes in the apomorphine-induced contralateral rotations. All animals that showed robust rotations after an acute low dose of apomorphine (0.05 mg/kg) 1 month after a nigrostriatal lesion continued to show the same level of rotations 3 months following the lesion. Although there was a reversal in the relative density of nerve terminal glutamate immunolabeling between the 1 and 3 month time period, this was not reflected in any change in dopamine agonist-induced rotations. It has been assumed that this type of locomotor behavior simply reflects the degree of striatal dopamine depletion and dopamine receptor up-regulation. However, we and others [31, 43] (but see [44]) have reported that a lesion of the frontal cortex or inactivation of

this area by local application of lidocaine [45] affects the number of apomorphine-induced rotations in animals with a nearly complete loss of striatal dopamine. Of particular interest in our study, and also reported by Ingham et al. [40], was that following a 6-OHDA lesion, there was a significant increase in the percentage of nerve terminals making an asymmetrical synaptic contact that contained a discontinuous, or perforated, postsynaptic density [30, 31]. This specialized type of glutamate synaptic contact has been suggested to be associated with enhanced synaptic transmission and has been seen to increase following the induction of long-term potentiation or after animals have been exposed to a complex environment [46, 47]. However, too many glutamate synapses containing a perforated postsynaptic density may be maladaptive. We reported that following ablation of the motor cortex in the 6-OHDA lesioned animals, there was a significant decrease in locomotor ability, as tested on a rotating rod (i.e., rotorod). However, not only was there a 50% increase in the number of synapses containing a perforated postsynaptic density but a similar increase in the number of nerve terminals making multiple synaptic contacts (i.e., multiple synaptic boutons) [48].

The striatum contains a significant level of the inhibitory neurotransmitter, gamma aminobutyric acid (GABA). It had been reported that using an antibody to the enzyme catalyzing the synthesis of GABA (glutamate decarboxylase, GAD), Nitsch and Riesenberg [49] found an increase in the percentage of GAD-immunoreactive boutons 6–8 months following a 6-OHDA lesion. These long-lasting changes in GABA neurochemistry are consistent with reports of an increase in GABA levels in patients with Parkinson's disease [50] and in animals following a 6-OHDA lesion [51–53]. Therefore, the relative density of this neurotransmitter within identified nerve terminals making a symmetrical synaptic contact was determined. We found that both 1 and 3 months following a nigrostriatal lesion, there was an increase in the density of nerve terminal GABA immunolabeling [30]. This would be consistent with increased GABA levels in the striatum following a lesion of the nigrostriatal pathway.

In Vivo Microdialysis

In order to correlate changes in the density of nerve terminal glutamate immunoreactivity with function, in vivo microdialysis was carried out in awake animals. Only those animals showing robust apomorphine-induced contralateral turning (>10 turns/min) were used. There was a significant increase in the basal extracellular level of striatal glutamate compared to the sham (control) group (Fig. 13.3).

The density of nerve terminal glutamate immunoreactivity was inversely associated with the extracellular level of striatal glutamate. The increase in extracellular striatal glutamate levels 1 month following a nigrostriatal lesion was associated with a decrease in the density of nerve terminal glutamate

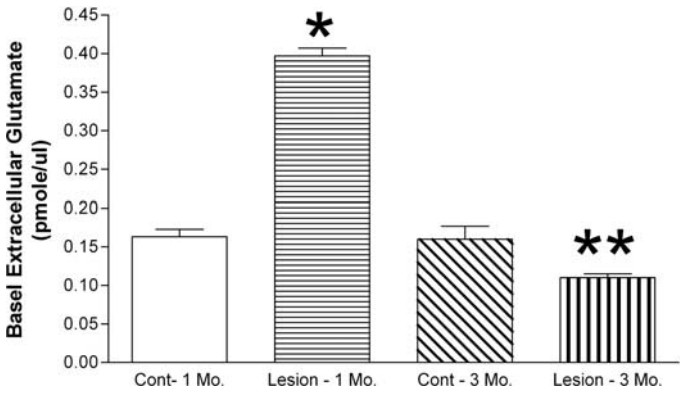

Fig. 13.3 Extracellular levels of striatal glutamate following loss of dopamine. 1 and 3 months following a nigrostriatal lesion, there is initially an increase and then a decrease, respectively, in the extracellular levels of striatal glutamate as measured by in vivo microdialysis. * – $p < .05$ compared to the 1-month control group. ** – $p < .05$ compared to the 3-month control group

immuno-gold labeling. As expected from the 3-month glutamate immunolabeling results, we find that there was a significant decrease in the extracellular level of striatal glutamate at this time period. These results are in agreement with the concept that an increase or decrease in the basal extracellular level of striatal glutamate compared to the sham group is inversely correlated with a decrease or increase in the density glutamate immuno-gold labeling within nerve terminals making an asymmetrical synaptic contact. Although the decrease in nerve terminal glutamate density at the 1-month time period could be due to a decrease in synthesis, this possibility was discounted since a decrease in synthesis would have resulted in a decrease in the basal extracellular level of glutamate. Just the opposite result was found (Fig. 13.3).

The increase in extracellular striatal glutamate 1 month following the loss of striatal dopamine is consistent with previous reports [30, 54–56] but inconsistent with others [57–60]. Why this discrepancy amongst the various reports is not known. However, the fact that we focus our microdialysis probes within the dorsolateral striatum may be important. This region of the striatum receives its input from the sensorimotor cortex [4], and all other regions of the striatum receive input from non-motor areas of the cortex. We have preliminary data that extracellular glutamate within the dorsolateral vs ventrolateral striatum are different, suggesting that probe placement may be even more critical than previously appreciated (McKee and Meshul, unpublished findings).

However, there is controversy as to the origin of the basal levels of glutamate that are measured in brain and whether this extracellular glutamate is derived from the (1) calcium-dependent vesicular pool, (2) calcium independent, cytoplasmic pool associated with the glutamate/cystine antiporter, or (3) glial pool

[61]. We and others have reported that about 30% of basal extracellular glutamate is calcium dependent [61–63] and that over 60% of the K^+-depolarized extracellular level of glutamate is calcium dependent [30]. This suggests a role for the synaptic vesicle pool within the nerve terminal contributing to the extracellular level of glutamate. Replacement of calcium with the divalent chelating agent, EGTA, and increasing the concentration of magnesium resulted in a decrease in the baseline level of glutamate [62], suggesting that a portion of the resting level of striatal glutamate is of neuronal and not glial origin. Of interest is our recent observation (Meshul et al., unpublished findings) that 3 months following a 6-OHDA lesion, the decrease in extracellular glutamate in the striatum is associated with an increase in the glutamate transporter protein, GLT-1, using Western immunoblotting. With an increase in GLT-1 protein, more glutamate would be transported into the glial cell, leading to the observed decrease in extracellular glutamate. However, we have also reported an increase in the density of nerve terminal glutamate immuno-gold labeling at this 3-month time period, suggesting that as glutamate accumulates presynaptically, less is being released [30, 62]. The increase in extracellular glutamate in the striatum 1 month following a 6-OHDA lesion suggests that the origin of the glutamate could be primarily derived from the nerve terminal, a finding consistent with our nerve terminal immuno-gold data. Although we have not measured GLTL-1 levels 1 month after a 6-OHDA lesion, the lesion-induced increase in extracellular glutamate may be due to reversal of the glutamate transporter [64, 65], increased release through a transporter-independent mechanism via the astrocytes [66] and/or cytoplasmic release via the cystine-glutamate antiporter [61].

It has also been reported that 60% of the basal extracellular level of glutamate is due to exchange with the glutamate/cystine antiporter, which has been shown to be calcium insensitive and most likely of cytoplasmic, but still of neuronal origin [61]. However, the relevance and contribution of the glutamate-cystine antiporter remains controversial [67]. Of major significance is whether there is sufficient extracellular cystine in order to stimulate the antiporter, since the suggested extracellular levels of cystine would provide minimal activation of the glutamate-cystine exchanger [68]. Furthermore, basal extracellular glutamate levels were reduced by 60% upon blockade of the cystine-glutamate antiporter with (S)-4-carboxyphenylglycine (CPG), which also blocks group 1 metabotropic glutamate receptors and activates group 2 metabotropic glutamate receptors. It has not been demonstrated whether CPG can attenuate glutamate release by the antiporter in the presence of a group I metabotropic glutamate receptor antagonist and a group II metabotropic glutamate receptor agonist. Therefore, the observed increase in striatal extracellular glutamate 1 month following a nigrostriatal lesion may be due to a combination of an increase in nerve terminal, astrocytic, or transporter release of glutamate.

Glutamate Plasticity in the Substantia Nigra Pars Reticulata

Another important area of the basal ganglia which receives a major excitatory (glutamate) input from the subthalamic nucleus, cortex, and pedunculopontine nucleus is the substantia nigra pars reticulata (SN-PR) [20]. The glutamate terminals originating from these brain areas make synaptic connections onto dopamine containing dendrites within the SN-PR [38, 68]. A decrease in glutamate output from the subthalamic nucleus reverses the behavioral effects of an MPTP lesion in monkeys and significantly decreases the bradykinesia/akinesia in humans [69, 70]. Microinjection of NMDA or AMPA glutamate antagonists into the SN-PR, decreasing the inhibitory GABAergic drive from the SN-PR to the motor thalamus results in enhanced behavioral activity in nigrostriatal lesioned animals [71]. A possible anatomical mechanism leading to this increased activity would be activation of the thalamo-cortico-striatal pathway. Dendrites from the overlying dopamine neurons in the SN-PC project down to the SN-PR, suggesting another site for dopamine–glutamate interactions [72, 73]. Therefore, glutamate synapses within both the SN-PR and the striatum play a critical role in Parkinson's disease.

In order to investigate glutamate plasticity within the SN-PR following the loss of striatal dopamine, in vivo microdialysis was carried out. In addition, low dose apomorphine, a dopamine D-1/D-2 agonist, was systemically injected for 7 days in order to induce locomotor sensitization and determine if this behavior results in further changes in SN-PR glutamate. We found that 1 month after the lesion, there was an increase in extracellular glutamate within the striatum, as previously reported [30] and apomorphine treatment resulted in a further increase in glutamate levels [39]. Within the SN-PR, a loss of striatal dopamine resulted in a decrease in extracellular glutamate, while apomorphine treatment leads to a further decrease in nigral glutamate. However, 3 months after a 6-OHDA lesion, there was a decrease in extracellular striatal glutamate, with apomorphine administration leading to essentially no further change in glutamate. The loss of striatal dopamine increased extracellular glutamate within the SN-PR while apomorphine administration resulted in a decrease in extracellular glutamate back to the value observed in the control group. This data suggested that the increase in striatal glutamate 1 month following a 6-OHDA lesion alone or following subchronic apomorphine is consistent with the hypothesis that a decrease in glutamate within the SN-PR leads to activation of the thalamo-cortico-striatal pathway. The decrease in striatal glutamate 3 months after a nigrostriatal lesion is also consistent with the observed increase in extracellular glutamate within the SN-PR, thus leading to a decrease in output of the thalamo-cortico-striatal pathway. These alterations in both striatal and SN-PR glutamate are consistent with the model of basal ganglia function as proposed by Albin et al. [20].

The increase in extracellular striatal glutamate in the dopamine-depleted striatum could be due to activation of the thalamo-cortico-striatal pathway. In agreement with this theory is the finding of an increase in cortical activity

within the sensorimotor cortex of rats following a 6-OHDA lesion compared to the sham (non-lesioned) group as measured by the BOLD (blood oxygenation level-dependent) method [74]. We hypothesized that increased activation of this pathway may be due to a decrease in glutamate within the SN-PR, leading to a decrease in GABAergic output from the SN-PR to the motor thalamus. We have reported that activation of the motor thalamus results in an increase in extracellular striatal glutamate [34] and that a lesion of the motor thalamus results in a decrease in striatal glutamate [75]. Indeed, we find that 1 month after a 6-OHDA lesion, there is a significant decrease in extracellular glutamate within the SN-PR compared to the sham group [39]. However, others have reported either no changes [58, 59, 76] or an increase in extracellular glutamate within the SN-PR in similarly nigrostriatal lesioned rats at the 1-month time period [57, 77–79]. The reason for this discrepancy is not apparent. Although we assume that the vast majority of the glutamatergic input to the SN-PR originates from the subthalamic nucleus [38], other afferents from the cortex and pedunculopontine nucleus may be making a smaller, yet important, contribution.

Within the SN-PR, administration of apomorphine to the lesioned group results in a significant decrease in extracellular glutamate compared to the lesioned group treated with saline. This should decrease the GABAergic output from the SN-PR to the thalamus, resulting in activation of the thalamo-cortico-striatal pathway, as predicted by the model. Since we actually observed a continued decrease in extracellular striatal glutamate in the lesioned group treated with apomorphine suggests that other factors may be controlling the extracellular level of glutamate. The glutamate transporter, GLT-1, is the major protein involved in the uptake of extracellular glutamate into glial cells [80]. It has been reported that following the administration of L-dopa in nigrostriatal lesioned rats for 21 days, there was a significant increase in expression of both protein and mRNA for GLT-1 within the striatum compared to the lesioned group treated with vehicle [81]. It is possible that in our study, treatment with apomorphine might also result in a similar increase in striatal GLT-1, leading to an increase in glutamate transport into glial cells and a decrease in extracellular glutamate. Such a decrease in extracellular striatal glutamate would be consistent with the findings in our 3-month study [30].

The time-dependent changes in striatal and SN-PR glutamate following a unilateral nigrostriatal lesion and the alterations in extracellular glutamate due to dopamine agonist treatment may be of interest in terms of behavioral changes associated with therapy used to treat patients with Parkinson's disease. The fact that extracellular glutamate levels are continuing to change over time in this nigrostriatal lesion model may complicate what effect dopamine agonists are having on glutamate within both the striatum and the SN-PR. If a similar degree of fluctuation of glutamate occurs in the Parkinson's disease patient, we speculate that the L-dopa- or dopamine agonist-induced behavioral changes that are observed in these patients could be due to continued fluctuations in glutamate within either the striatum or SN-PR.

Alterations in Glutamate in the Subthalamic Nucleus

It has been reported that loss of dopamine within the SN-PC, as seen in Parkinson's disease, results in an increase in activity of the subthalamic nucleus (STN) [82–86], the only area within the basal ganglia whose output is excitatory [20]. This finding is consistent with the model of basal ganglia function as predicted for Parkinson's disease [20]. The STN is of particular importance in the circuitry of the basal ganglia since either a lesion or high-frequency stimulation (i.e., deep brain stimulation) of the STN in humans with Parkinson's disease or in the non-human primate model results in a reduction in many of the behavioral deficits associated with this disease [69, 87–89]. The model of basal ganglia function would predict that the increase in STN activity could be due to a decrease in GABAergic inhibitory input from the external globus pallidus to the STN, a finding consistent with a recent report using the monkey model of Parkinson's disease [90]. This finding of a decrease in GABA in the STN is consistent with the model of basal ganglia function [20]. Of significance was that following MPTP, the increase in STN neuronal bursting was not mimicked by simply a lesion of the external globus pallidus, which provides the GABA input to the STN [90, 91]. This suggests that although the loss of nigrostriatal dopamine leads to a decrease in extracellular GABA in the STN, other neurotransmitters, such as glutamate, may be influencing the activity of the STN neurons. However, there are several sources of excitatory, glutamatergic input to the STN [92], including those pathways from the cortex, thalamus, and pedunculopontine nucleus (PPN) [93, 94], that have not been investigated in the STN following the loss of dopamine. This later input from the PPN also contains acetylcholine (ACh). There is an enrichment of ACh and glutamate-containing nerve terminals making an asymmetrical vs symmetrical synaptic contact [95]. At least within the striatum, the input from cortex and thalamus can be distinguished using a marker for the vesicular transporters: vesicular glutamate transporter 1 or 2 (VGLUT-1 and VGLUT-2), respectively [5, 6, 96]. Ultrastructurally within the STN, VGLUT-1-labeled terminals are observed making axospinous synaptic contact (Fig. 13.4) and VGLUT-2-labeled terminals from the thalamus that originate from midline, dorsomedial, and ventral anterior/lateral nuclei also make axospinous synaptic contact. The VGLUT-2-labeled terminals from the parafasicular/centromedian nucleus are observed making axodendritic contact [5–7].

Terminals labeled for the enzyme associated with ACh synthesis, choline acetyltransferase (ChAT), are shown to co-label for the neurotransmitter, glutamate, and are making an asymmetrical synaptic contact onto a dendritic spine (Fig. 13.5).

The STN is of particular interest in Parkinson's disease since a decrease in the glutamate output from this nucleus reverses the behavioral effects of an MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) lesion in monkeys and significantly decreases the bradykinesia/akinesia in humans [69, 70]. In addition,

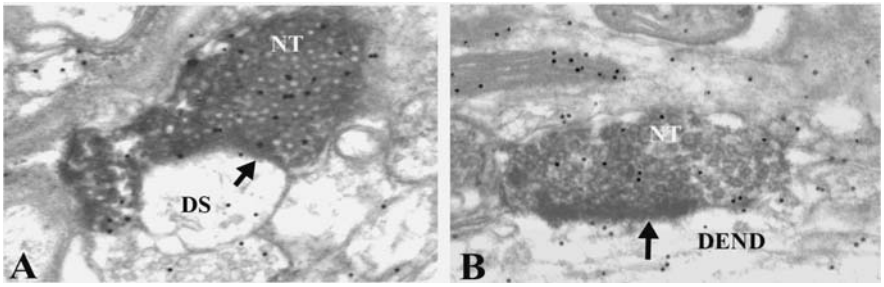

Fig. 13.4 Electron photomicrographs of double immunolabeling for VGLUT-1 (**A**) or VGLUT-2 (**B**) (dark reaction product inside the nerve terminal) and glutamate immuno-gold (note 10 nm gold particles inside the nerve terminal, similar to that seen in Fig. 13.1) within the STN. The VGLUT-1/glutamate immuno-gold-labeled terminal in A is making an asymmetrical synaptic contact (*arrow*) with a dendritic spine (*DS*) and that the VGLUT-2/glutamate immuno-gold-labeled terminal is making synaptic contact (*arrow*) with a dendrite (*DEND*)

blockade of the glutamate input to the internal globus pallidus, primarily from the STN, has been observed to alleviate Parkinsonism in animals and humans [21, 97, 98]. Although there appears to be conflicting data regarding the activity of the external globus pallidus and its role in influencing the STN [99], there is evidence suggesting that the inhibitory GABAergic input from the globus

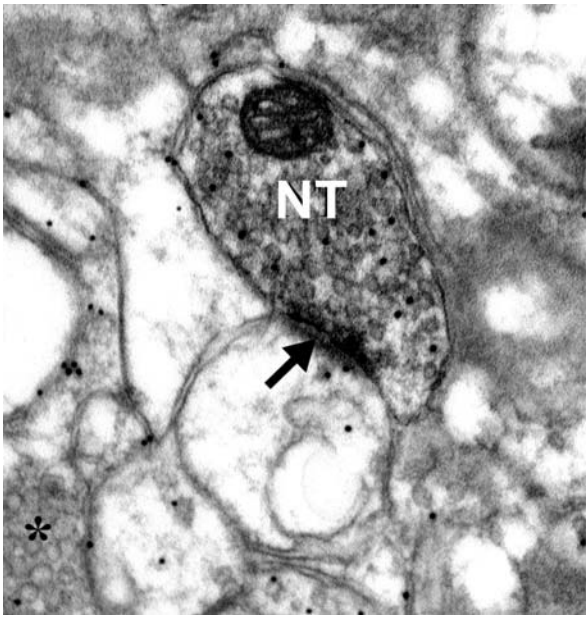

Fig. 13.5 Electron photomicrograph of double immunolabeling for choline acetyltransferase (ChAT) [dark reaction product inside nerve terminal (*NT*) primarily surrounding the synaptic vesicles] and glutamate immuno-gold (note 10 nm gold particles inside the nerve terminal). The ChAT-labeled nerve terminal is making an asymmetrical synaptic contact (*arrow*) with an underlying dendritic spine. Part of an unlabeled nerve terminal containing synaptic vesicles (*asterisk*) is seen in the lower left corner, demonstrating the specificity of the ChAT immunolabeling technique

pallidus to the subthalamic nucleus, resulting in membrane hyperpolarization, is involved in regulating the bursting activity of the STN [100, 101].

We have recent evidence that the loss of dopamine from the substantia nigra results in an increase in the basal extracellular level of glutamate within the STN, a finding that has never been reported. In animal models of nigrostriatal degeneration, and in humans with Parkinson's disease, administration of glutamate receptor antagonists decreases the parkinsonian symptoms associated with the loss of striatal dopamine and decreases the L-dopa-induced dyskinesias [21, 23, 25–27, 102]. Since the glutamate receptor antagonists are administered systemically, it is possible that blockade of glutamate receptors within the STN is an important site of action. Therefore, glutamate synapses within the STN may play a central role in the development of the movement disorders associated with Parkinson's disease.

We find that in an animal model where the nigrostriatal pathway has been injected with the neurotoxin, 6-OHDA, there is a nearly complete loss of striatal dopamine, with no recovery, over a 3-month time period. This results in an increase in the extracellular levels of glutamate within the STN (Fig. 13.6B). This increase in extracellular glutamate levels could then activate the STN, a finding that would be consistent with the model of basal ganglia function [20]. After the loss of dopamine, we find a decrease in STN glutamate immuno-gold labeling within the synaptic vesicle pool of the nerve terminal. This suggests that an increase in the extracellular levels in the STN, which can be interpreted as enhanced release of glutamate, is associated with a decrease in nerve terminal glutamate labeling. We previously reported of such an inverse

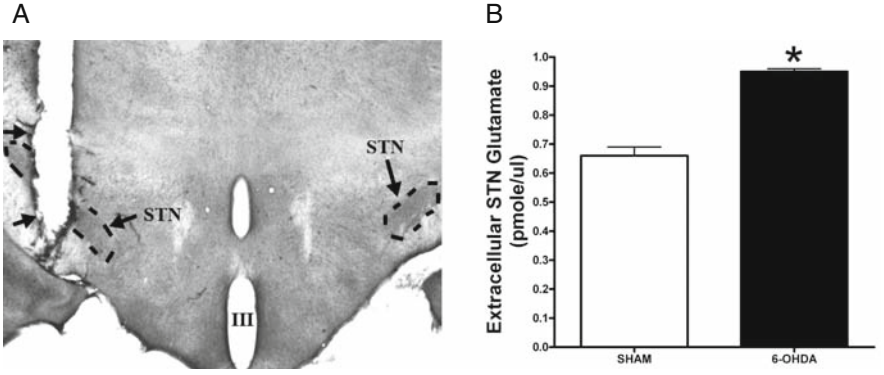

Fig. 13.6 (**A**) Placement of 1 mm dialysis probe into the STN. The 1 mm tip of the probe, located on the left side of the brain between the two *arrows*, is shown located in the middle of the left STN. The *dotted lines* on the left side show the location and extent of the STN. The STN on the right side of the brain is also illustrated. The cerebral peduncles (*white matter*) are located just below the STN. (**B**) Baseline extracellular level of glutamate within the STN following a unilateral lesion of nigrostriatal pathway. Note the significant increase in glutamate levels in the 6-OHDA lesioned group compared to the sham group. Values are means ± S.E.M. * – $p < .05$ using the Student's t-test

association between extracellular glutamate levels and the density of glutamate immuno-gold labeling within striatal nerve terminals making an asymmetrical (excitatory) synaptic contact [30, 75].

The largest change in nerve terminal glutamate immuno-gold labeling following a 6-OHDA lesion occurs in those terminals making an axospinous vs axodendritic contact. We find that these terminals contact on spines are primarily labeled with the vesicular glutamate transporter-1 (*VGLUT-1,* Fig. 13.4a) as opposed to the vesicular glutamate transporter-2 (*VGLUT-2,* Fig. 13.4b) (Table 13.1). Since the primary origin of the VGLUT-1 containing neurons is the cortex [37, 96], these data suggest that alterations in glutamate synapses within the STN following the loss of striatal dopamine are associated with at least the cortico-subthalamic pathway. This finding is consistent with the theory of a hyperdirect pathway from cortex to STN [103].

There was nearly a 20% decrease in the density of glutamate immuno-gold labeling associated with the synaptic vesicle pool for all an asymmetrical synaptic contacts in the lesioned compared to the sham group (Table 13.1: 84.5 vs 70.0). However, this difference was not statistically significant. Within the STN there are nerve terminals making synaptic contact with both dendritic spines and shafts [104]. When the density of nerve terminal glutamate immuno-gold labeling was determined separately for spine and shaft synaptic contacts, the difference in labeling was primarily associated with nerve terminals making an axospinous synaptic contact (Fig. 13.4A). Overall, there was a significant decrease in the density of nerve terminal glutamate immunolabeling associated with the synaptic vesicle pool in the 6-OHDA lesioned compared to the control group (95.4 vs 77.6, Table 13.1). Not only was there a decrease in the number of immuno-gold particles per nerve terminal in the lesioned vs control group, but the size of the terminal making an axospinous contact was smaller following the loss of dopamine vs the non-lesioned group (Table 13.1, Spine Synapses).

Due to the controversy associated with the origin of extracellular glutamate, extracellular glutamate levels can also be influenced by either reversal of the glutamate transporter [64, 65] or release through a transporter-independent mechanism via the astrocytes [66]. Therefore, the observed increase in extracellular glutamate in the STN following a nigrostriatal lesion may be due to a

Table 13.1 Morphological measurements of STN glutamate synapses

Group	Glutamate density # gold particles/μm^2 (mean ± SEM) *All terminals*	Shaft synapses (Glu density) # gold particles/ μm^2(mean ± SEM)	Spine synapses (Glu density) # gold particles/ μm^2(mean ± SEM)	Spine synapses (area, μm^2)	Spine synapses (# gold particles/ terminal)
Sham	84.5 ± 7.3	73.2 ± 5.5	95.4 ± 3.4	0.78 ± .02	74.4 ± 3.8
6-OHDA	70.0 ± 5.8	67.6 ± 5.3	77.6 ± 3.1* *-vs CTL (*-$p < .05$)	0.64 ± .03* *-vs CTL (*-$p < .05$)	49.7 ± 4.5* *-vs CTL (*-$p < .05$)

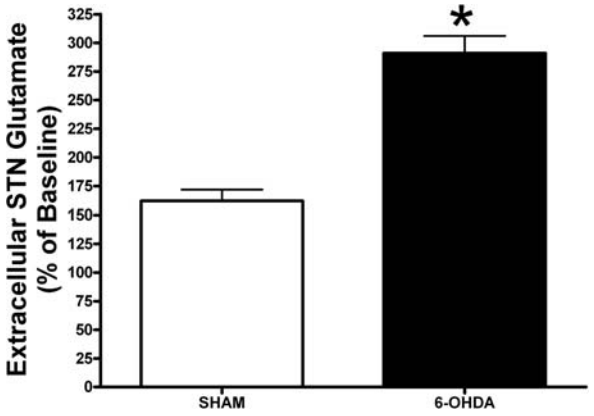

Fig. 13.7 Extracellular level of glutamate within the STN following the infusion of the glutamate transporter blocker, PDC. Baseline samples were collected and then PDC (1 mM) was infused through the probe and an additional eight 15 min samples collected. Although both the sham and 6-OHDA groups show an increase in extracellular glutamate following PDC infusion, there was a much larger increase in the 6-OHDA compared to the sham group. Values are mean % ± S.E.M. * – $p < .05$ using the Student's t-test

combination of an increase in nerve terminal, astrocytic or transporter release of glutamate. In order to test this, an inhibitor of the glutamate transporters, L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC) [105], was infused into the STN by reverse microdialysis, resulting in a greater increase in extracellular glutamate in the lesioned compared to the sham group (Fig. 13.7). It has been reported that PDC not only blocks glutamate transporters but that 50% of the increase in extracellular glutamate is inhibited by either the addition of tetrodotoxin or decreasing the levels of calcium within the artificial cerebrospinal fluid [105]. Therefore, at least half of the PDC-induced increase in extracellular glutamate is due to synaptic release of this neurotransmitter.

Could there be a change in the levels of the major glutamate transporter, GLT-1, within the STN following a nigrostriatal lesion? We find that following a 6-OHDA lesion, there is a significant increase in the relative optical density of the glutamate transporter, GLT-1 (+320%) and an increase in glial fibrillary acidic protein (GFAP) protein levels (+53%) compared to the sham group by western immunoblotting (Fig. 13.8). The GLT-1 data suggest that with increased synaptic activity, as reported by others (see review [93]), and increased extracellular levels of glutamate in the STN (Fig. 13.6) following the loss of dopamine, there is a compensatory increase in the protein levels of this transporter located on glial cells. Alternatively, there could be reversal of the transporter, resulting in an increase in release of glutamate from the glial cells (hence, the increase in GFAP) into the extracellular space, a finding consistent with our dialysis data (Fig. 13.6) [64–66]. These data suggest that the greater PDC-induced increase in extracellular glutamate in the 6-OHDA lesioned vs

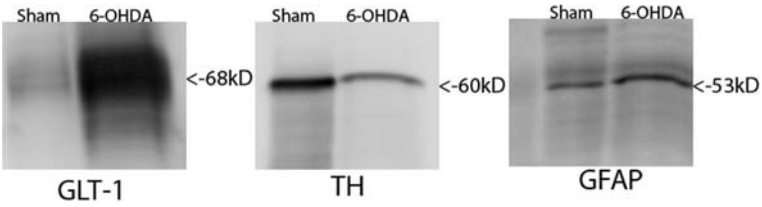

Fig. 13.8 Effect of a 6-OHDA lesion of the nigrostriatal pathway on changes in the relative optical density for GLT-1, TH, and GFAP protein within the STN. Using Western immunoblotting, there was a significant increase in the protein level for GLT-1, a significant decrease in TH protein, and a small increase in GFAP protein in the 6-OHDA lesioned group compared to the sham group

sham group may be due to increased levels of the GLT-1 transporter. But the decrease in nerve terminal glutamate immunolabeling in terminals making an axospinous contact is consistent with increased synaptic release of glutamate.

However, can targeted drug delivery into the STN that influences the glutamatergic output of this nucleus be effective in terms of therapy for Parkinson's disease [106, 107]? We find that acute infusion into the STN of the NMDA antagonist, MK-801, leads to a decrease in STN extracellular glutamate (Fig. 13.9) and a significant increase in the use of the affected forepaw (Table 13.2). This suggests that targeted drug delivery of a glutamate receptor antagonist into the STN can lead to an increase in motor function that is associated with a decrease in extracellular glutamate.

These data are consistent with the model of basal ganglia function, in which the loss of striatal dopamine leads to an increase in STN activity and blockade of STN glutamate receptors would lead to an increase in locomotor function. These data are of interest since it was reported that injection of an AMPA or

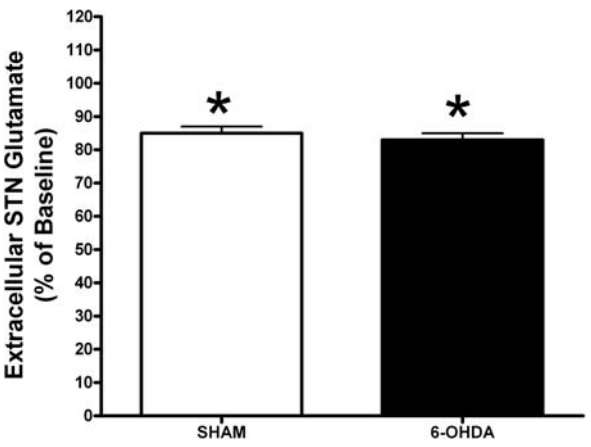

Fig. 13.9 Extracellular level of glutamate within the STN following the infusion through the probe of the non-competitive NMDA glutamate receptor antagonist, MK-801 (50 mM). Baseline samples were collected and then MK-801 was infused through the probe and an additional eight 15 min samples collected. Values are means % ± S.E.M. *$p < .05$ using the Student's t-test compared to baseline values

Table 13.2 Use of affected forepaw during the infusion of MK-801 into the STN

Group	% Forepaw extensionsprior to MK-801 infusion	% Forepaw extensions after MK-801 infusion
6-OHDA	3 ± 2.2	40 ± 5.1

metabotropic glutamate agonist, but not an NMDA agonist, into the STN resulted in parkinsonian rigidity [29]. This suggests that agonists and antagonists may be acting differently in the STN in terms of their affect on locomotor movement. In addition, in humans with Parkinson's disease, acute targeted drug delivery of either lidocaine, to block impulse-dependent activity, or muscimol, a GABA-A receptor agonist, improved the motor symptoms [108]. Therefore, blocking the activity of the STN improved the bradykinesia, limb tremor, and rigidity associated with this disease. Targeted drug delivery of neurotrophic factors infused into the putamen of Parkinson's patients is currently undergoing clinical trials with positive outcomes, suggesting that future drug therapy may involve direct application of compounds into specific brain regions [109]. It has been reported that systemic injection of an AMPA antagonist in the monoamine depleted mice or the MPTP-treated monkey resulted in an increase in motor function [25]. A lesion of the PPN reduced the hyperactivity of the STN in the 6-OHDA rat model, while deep brain stimulation of both the STN and the PPN in patients with Parkinson's disease provided further motor improvement [110, 111]. This suggests the importance of the glutamate/acetylcholine input to the STN. In animal studies, there have been several other reports that local injection of either an NMDA antagonist [112], muscarinic antagonist [113], GABA-A agonist [98] or a metabotropic glutamate receptor type 5 antagonist [114] into the STN improved motor function in either rats or monkeys. These studies suggest that targeted drug delivery into the STN can improve motor movement in animal models and in humans with Parkinson's disease.

Overall Conclusion

The loss of nigrostriatal dopamine results in alterations in glutamate within several regions of the brain. Although the striatum has been the primary focus of numerous studies involving glutamate function, other key areas associated within the basal ganglia that have been studied in terms of glutamate alterations in these animal models are the substantia nigra and subthalamic nucleus. For the most part, consistent with the model of basal ganglia function [20], we have found that long-term loss of striatal dopamine results in an increase in glutamate in the subthalamic nucleus, substantia nigra pars reticulata, and a decrease in the striatum. Current surgical therapy for Parkinson's patients includes, in some cases, deep brain stimulation of the STN. Regardless of the mechanism of this type of stimulation in terms of its effectiveness in treating some of the

symptoms of Parkinson's disease, we have recent data suggesting that targeted drug infusion of a glutamate receptor antagonist into the STN not only decreases the extracellular level of glutamate in this brain region but also increases the use of the affected forepaw. Therefore, the control of glutamate activity within at least the STN may prove to be the most effective site in terms of drug therapy.

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Part III
Computational Analyses of the Cortico-Subcortical Dynamics and Parkinson's Disease

Chapter 14
Neuromodulation and Neurodynamics of Striatal Inhibitory Networks: Implications for Parkinson's Disease

Tomomi Shindou, Gordon W. Arbuthnott, and Jeffery R. Wickens

Abstract Anatomists suggested many years ago that inhibitory interactions among the spiny projection neurons of the striatum are very probable and might give rise to a lateral inhibition type of neural network. Inhibitory interactions have since been confirmed by direct electrophysiological measurement using dual intracellular or whole cell recordings from pairs of spiny projection neurons. Inhibitory interactions have the potential to produce competitive dynamics among spiny projection neurons, though the importance of this role is debated because lateral inhibition is sparse and weak at the level of individual synaptic connections. On the other hand, at the population level, network simulations using realistic values for synaptic connectivity and efficacy support the concept of the striatum as a dynamic focussing device based on lateral inhibition. This operation may be important for normal function of the striatum in the selection of motor programs. Previously, it has been shown that neuromodulators, such as dopamine, modulate lateral inhibition in the striatum. In Parkinson's disease, loss of dopaminergic modulation of lateral inhibition may alter striatal dynamics and contribute to some of the symptoms of Parkinson's disease. Therefore, modulators that restore striatal dynamics may have therapeutic potential. Recently, we have shown that adenosine A2A receptors increase lateral inhibition in the striatum, an effect mediated by presynaptic modulation of spiny neuron terminals. These receptors are of particular interest in view of the therapeutic effects of adenosine A2A receptor antagonists in Parkinson's disease. Here we summarise our current thinking about lateral inhibition in the striatum, its modulation by adenosine A2A receptors, and its significance for the therapeutic effects of A2A receptor antagonists in Parkinson's disease.

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Introduction

The striatum is the principal input structure of the basal ganglia. Numerically, the great majority of neurons in the striatum are spiny projection neurons, which produce the inhibitory output of the striatum to the globus pallidum and substantia nigra. The major glutamatergic afferents to the striatum from the cerebral cortex make monosynaptic contact with spiny projection neurons. The dopaminergic afferents from the substantia nigra also synapse directly on the spiny projection neurons. Thus, the spiny projection neurons play a crucial role in the input–output operations of the striatum by integrating glutamatergic cortical inputs with dopaminergic inputs and producing the output to other basal ganglia nuclei.

Anatomical observations made nearly 30 years ago suggested that inhibitory interactions among the spiny projection neurons of the striatum are very probable. Individual spiny projection neurons produce a local axonal plexus in the spheroidal space occupied by their own dendritic trees [1, 2]. Based on the GABAergic nature of these neurons and their synaptic contacts with other spiny neurons, several authors have proposed that the spiny projection neurons form a lateral inhibition type of neural network [3–5]. In the idealised concept of lateral inhibition, each output neuron makes inhibitory synaptic contact with its neighbours [5]. However, there are physical limitations set by the extent of axonal and dendritic trees, and the number of synaptic sites, which mean that lateral inhibition is limited to a local domain of inhibition. In the striatum such local domains are probably not physically compartmentalised, but may arise as a dynamic property of local inhibitory interactions across a continuous network of connections [6].

Recently, this idealised concept of lateral inhibition by spiny projection neurons striatal circuit has required modification in light of new findings. Quantitative anatomical considerations show that the connectivity among spiny projection neurons must be sparse, and that not every neuron contacts every other, even within spatially limited domains [7–9]. This sparse connectivity means that the connections between pairs of spiny projection neurons are not likely to be reciprocal, unless there are special controls over growth to select for such connections [10–13]. Importantly for dynamical considerations, the cortical input has also been shown to be sparsely distributed, with adjacent neurons receiving few inputs in common [14]. This has led to a more accurate concept of the striatal spiny cell network in which the spiny cells form a sparsely connected network in which a small fraction of neighbouring cells are connected (on the order of 20%) and the individual connections involve one or very few synaptic contacts.

In addition to changes in our concept of the local interactions among the spiny projection neurons, much has been learnt about the anatomical and physiological properties of several types of GABAergic interneurons in the striatum [15–17]. Although these are present in relatively small numbers, in

that the feedforward interneurons are outnumbered almost 100 to 1 by the spiny projection neurons, nevertheless each fast-spiking interneuron exerts a disproportionately large influence [16, 17]. Individually, a fast-spiking interneurons makes on the order of ten times more synaptic contacts than a single projection neuron [18]. The majority of their synaptic connections are on the soma of the spiny projection neurons, where they are in a position to have a strong effect on spike firing [19]. They are also, as their name suggests, relatively easy to excite to fire at high frequencies. Therefore, it is likely that individual FS interneurons have a strong influence over the neurons that they contact.

The relative importance of FS interneurons and spiny projection neurons at the network level is difficult to determine because it depends on the fraction of cells that are firing at any time, and their rates of firing. If a large fraction of the spiny projection neurons is active, the effects of the spiny projection neuron collaterals will dominate. At the time of writing it is not known what fraction of the spiny projection neurons or FS interneurons is active during any behaviour, including quiet rest. The actual number of spiny neurons that are active during a particular movement is difficult to determine without definitive identification of the neurons and measurements of the number of firing and non-firing cells in awake animal experiments. The differences between inhibition mediated by FS interneurons and spiny projection neurons have been reviewed recently. In the present chapter we focus on the spiny projection neuron network and its modulation by adenosine A2A receptors.

Electrophysiology of Inhibitory Interactions Between Spiny Projection Neurons

Direct electrophysiological measurement using dual intracellular or whole cell recordings from pairs of spiny projection neurons has confirmed the existence of lateral inhibition in the striatum. Using sharp electrodes to make dual intracellular recordings from pairs of spiny neurons in striatal brain slices, Tunstall et al. [20] found nine connected cells in a sample of 45 pairs of spiny projection neurons, corresponding to a probability of 0.1. With improved sensitivity for detecting a connection, we have detected connections with a slightly higher probability. In a recently reported study [21] we detected GABAergic synaptic connections in 29% of a sample of 194 pairs of spiny projection neuron, which were separated by distances ranging from 46 to 140 μm (89 ± 4 μm, $n = 35$). Reciprocal connections were rare, occurring in only 7% of connected pairs (4 of 56 pairs). The probability of a connection existing between spiny projection neurons was estimated from this data to be 0.15 (60 of 388 chances). These figures are compatible with those obtained using similar techniques in other laboratories [15, 22, 23]. A higher connection probability ($p = 0.25$) has been detected in organotypic slice cultures [17, 24].

The efficacy of the spiny cell-spiny cell synapse has been measured by several different groups. The amplitude of the IPSCs we observed in our sample of connected pairs was 22 ± 4 pA (n = 41). Using mean-variance analysis Koos et al. [15] determined a peak quantal conductance change at spiny–spiny synapses from 0.3 to 0.9 nS. They reported also that the use of intracellular Cs^+ increased their estimate of the mean synaptic conduction for this connection by a factor of three, implying attenuation of apparent synaptic currents at distant synapses by the electronic properties of the spiny cell dendrites. These values are consistent with the very small number of synapses per connection suggested by quantitative anatomical considerations [9].

Competitive Dynamics Among Spiny Projection Neurons

Early models of lateral inhibition in the striatum assumed strong inhibitory synapses and high probability of synaptic connection. Such models produced a competitive "winner-take-all" dynamic, in which the most active neurons suppressed activity in the less active neurons [5, 10, 25–29]. As detailed in the previous section, physiological measurements showed that synaptic currents were an order of magnitude less than had been assumed previously and the probability of synaptic connections was much lower. These observations raised doubts about the potential for competitive dynamics, because lateral inhibition is sparse and weak at the level of individual synaptic connections.

Analyses of the effects of one single spiny neuron on another are only a part of the equation. Another important measure is population activity of the striatal network, because the effects of individual synaptic interactions summate. It is important to determine whether the number of active inputs is sufficient to produce an effect on the postsynaptic cell, given the effect of a single synapse. In order to relate the results of pairwise studies to the activity of the striatal network in an awake, behaving animal, it is therefore necessary to know the fraction of spiny cells that are active in a given period. It has been shown that the majority of the spiny projection neurons do not fire action potentials when the animal is at rest [30]. However, it is unclear what fraction of cells become active when the animal is moving, because single unit recording does not provide information about the cells that remain inactive.

In view of the difficulty of obtaining direct measures, we have used a computational approach to examine the implications of the data from pairs for the activity of larger networks with anatomically realistic connections. We developed a simple model of a small block of striatal tissue and simulated action potential firing in response to cortical inputs. By turning the spiny–spiny inhibitory interconnections on and off, we were able to see what effect they had on overall network activity. An example of the findings is shown in Fig. 14.1. Details of the simulation can be found elsewhere [31]. The results suggest that under natural firing conditions in the striatal network as a whole, lateral inhibition produces strong effects on the overall activity levels.

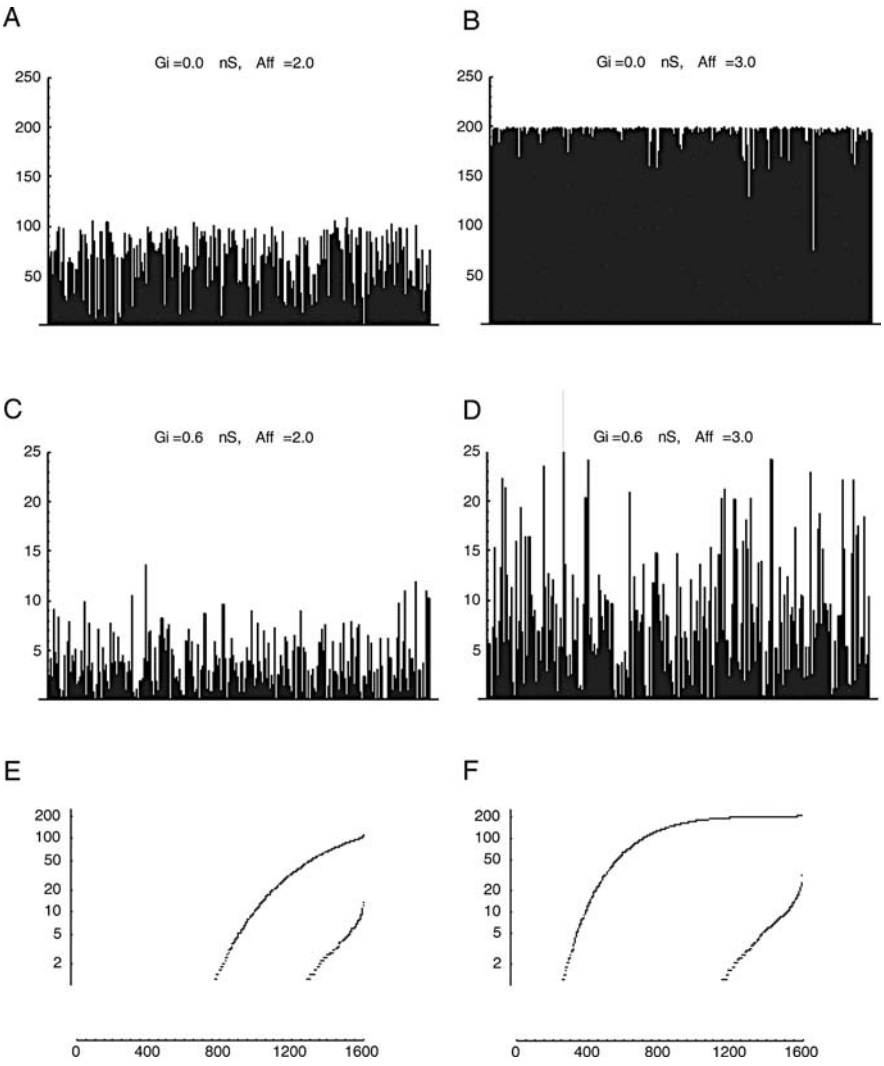

Fig. 14.1 Results of computer simulation based on realistic estimates of connectivity and synaptic conductance. **A**, **B**. Average firing rates of all cells in a 2500 cell network, with zero inhibitory interactions and an average excitatory afferent rate of (A) 2000 Hz, corresponding to 10 Hz action potential firing in 200 active inputs or (B) 3000 Hz, corresponding to 10 Hz action potential firing in 300 active inputs. **C**, **D**. Firing rates in same network as in A and B, but with inhibitory synapses (Gi = 0.6 nS) formed with probability of p = 0.2, giving about 500 inhibitory inputs per cell. **E**, **F**. Comparison of distribution of firing activity (cells arranged in order of excitatory input) showing effects of inhibition as shown in **A**–**D**. Note that the presence of physiological levels of feedback inhibition the difference between less and more active cells is increased, showing functional competitive dynamics in the network. Adapted from model described by Wickens et al. [31]

Neuromodulation of Lateral Inhibition by Dopamine and Adenosine

Many pieces of evidence suggest that inhibition mediated by spiny projection neurons is regulated by dopamine [24, 32–34]. Surround inhibition between spiny projection neurons is increased by activation of D1 receptors whereas activation of D2 receptors decreases surround inhibition [33–36]. On the other hand, GABA(A) receptor-mediated currents are reported to be reduced by D1 receptor agonists [37]. When observed at the level of individual presynaptic terminals, the effects of dopamine may vary from terminal to terminal [38, 39] suggesting variations in expression of dopamine receptors on individual terminals. These results await confirmation in further studies, which may lead to a more coherent account, but collectively they suggest that dopamine modulates lateral inhibition in the striatum.

If dopamine modulates lateral inhibition, it seems likely that loss of dopaminergic modulation of lateral inhibition would occur in Parkinson's disease, and this in turn may alter striatal dynamics. For example, vGAT expression is altered in the 6-hydroxydopamine model of Parkinson's disease [40]. We speculate that loss of dopaminergic modulation of lateral inhibition may contribute to some of the symptoms of Parkinson's disease. Therefore, modulators that restore striatal dynamics may have therapeutic potential as non-dopaminergic treatments for Parkinson's disease.

There is growing evidence that adenosine is a critical modulator of specific basal ganglia output pathways via the adenosine A_{2A} receptor (for reviews, see [41, 42]). Thus, adenosine is potentially of comparable importance to dopamine in normal basal ganglia function and in the pathophysiology and treatment of Parkinson's disease [43].

At the cellular level, mRNA for adenosine A_{2A} receptors is enriched in striatal neurons [44–46]. Importantly, adenosine A_{2A} receptors are expressed in indirect pathway cells [44] but not in direct pathway cells [47]. Thus, adenosine A_{2A} receptors are selectively associated with spiny projection neurons of the indirect, striopallidal pathway. It has, therefore, been considered a potentially important circuit in the pathophysiology of Parkinson's disease [41, 42].

At the ultrastructural level, adenosine A_{2A} receptors are colocalised with GABA in axon terminals and dendrites [48]. Thus, the presynaptic terminals of spiny projection neurons, and the postsynaptic dendrites are key sites at which adenosine A_{2A} receptors may regulate the output of these neurons. Consistent with their presynaptic location, adenosine A_{2A} receptors modulate the release of GABA from collaterals of spiny projection neurons [41, 49]. In brain slice patch-clamp electrophysiological studies, adenosine A_{2A} agonists reduce, and antagonists enhance, evoked GABAergic inhibitory postsynaptic currents (IPSCs) [49]. A presynaptic locus of these effects of adenosine is supported by a similar regulation by adenosine A_{2A} receptors of spontaneous miniature IPSCs, but not of responses to directly applied GABA. However, since field

stimulation was used in these studies, the presynaptic neurons could not be identified as direct or indirect pathway cells. It is also possible that GABA interneurons contributed to these effects.

We have recently extended these findings using paired whole cell recordings, which enabled the presynaptic neurons mediating the effects of adenosine A_{2A} receptors on inhibitory interactions to be identified definitively (Fig. 14.2). In these experiments we found that A_{2A} receptors facilitate GABAergic

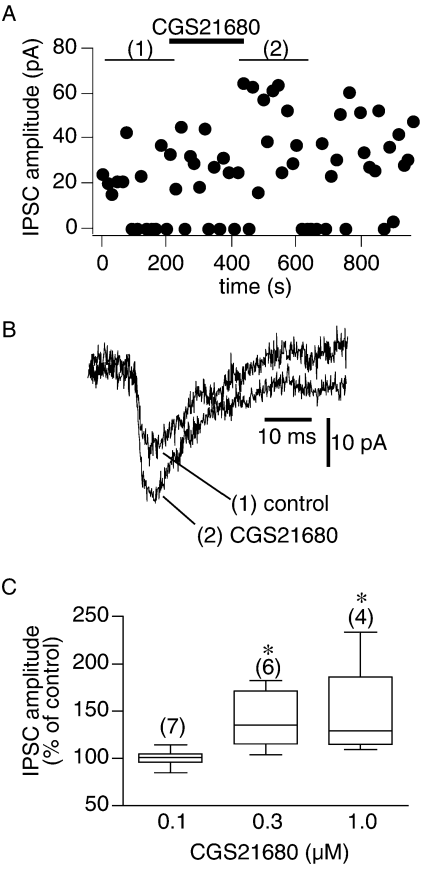

Fig. 14.2 Enhancement of inhibitory interactions between spiny projection neuron pairs by an adenosine A_{2A} receptor agonist. (**A**) Time course of the amplitude of IPSCs between spiny projection neuron pairs during application of an adenosine A_{2A} receptor agonist, CGS21680 (1 μM). (**B**) Spiny projection neuron IPSCs before (control) and during application of CGS21680, each superimposed trace is an average of 15 responses. (**C**) Summary of CGS21680-induced IPSCs of spiny projection neuron pairs. Data are normalised values expressed as a percentage of control values. Box plots show interquartile range, median, and whiskers show maximum and minimum values. The number of pairs examined is given in parentheses. *$P<0.05$ vs. control by paired t test. Reprinted from Shindou et al. [21] and used with permission of the publisher

interactions between spiny projection neurons in the neostriatum [21]. This effect was presynaptically mediated, as evidenced by changes in the paired-pulse ratio and failure rate that accompanied facilitation of IPSCs. We also obtained immunohistochemical evidence for A_{2A} receptors on the intrastriatal terminals of the neurons in the study. These results provided direct evidence that A_{2A} receptors facilitate lateral inhibition in the neostriatum. This facilitatory effect is consistent with a previous report showing presynaptic A_{2A} receptors increase GABA release from striatopallidal terminals in the globus pallidus [50], one of the major targets of the neostriatal projection neurons. Together with the present findings this indicates that A_{2A} receptors positively modulate GABAergic synaptic transmission at both neostriatal and pallidal terminals of the neostriatal projection neurons.

Conclusion

It is now well established that the GABAergic synapses between spiny projection neurons are electrophysiologically functional. The connections between individual pairs of neurons are relatively sparse and weak, but collectively these synapses make up the majority of GABAergic synapses in the striatum. Under in vivo conditions, this lateral inhibition may produce competitive dynamics, such that more active groups of neurons suppress the activity of less active cells. This limits the number of spiny projection neurons that can become active at any one time. Many pieces of evidence indicate that lateral inhibition is modulated by dopamine, though there appears to be considerable variation in the direction of this effect. In Parkinson's disease, loss of dopaminergic modulation of GABAergic synapses may lead to changes in striatal neurodynamics. We have recently found that adenosine A2A receptors facilitate lateral inhibition in the striatum, by a presynaptic action on the terminals of spiny projection neurons. This action of adenosine A2A receptors may account for some of the therapeutic effects of adenosine A2A receptor antagonists in Parkinson's disease.

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Chapter 15
Dopaminergic Modulation of Corticostriatal Interactions and Implications for Parkinson's Disease

John A. Wolf and Jason T. Moyer

The striatum includes the structures of the caudate, putamen, and nucleus accumbens (Nacb) and is a subset of the subcortical interconnected nuclei that make up the basal ganglia [39]. The striatum is one of the major input structures of the basal ganglia and integrates input from many cortical areas as well as the thalamus. In general, the dorsal striatum processes more input from the motor regions of the cortex, while the ventral striatum processes input predominantly from the limbic and cognitive areas of the brain [2].

The predominant cell in the striatum is the GABAergic medium spiny neuron (MSN), comprising over 95% of the striatal cells in the rodent [39]. The other 3–5% of cells in the striatum are GABAergic and cholinergic interneurons, each of which plays an important role in striatal function [38]. While each MSP cell has a small soma (10–15 μM), it has a large dendritic structure (200–300 μM) with numerous spines, and a branching axonal segment. The branching axonal arbor can be larger than the dendritic arbor, makes lateral contacts with other MSNs, and has one portion of the axonal segment projecting to efferent structures. The processing of afferent input by these MSNs has been the focus of numerous investigations, as has the role of dopaminergic (DA) modulation in affecting the integration of both inputs and the output of the MSNs [6, 32, 27, 31].

MSNs have a bimodal membrane potential under anesthesia or in vitro (see below), and one of the critical questions about their processing of cortical input has been whether they can maintain their depolarized state via intrinsic mechanisms without any further synaptic input. We have addressed some of the issues associated with cortical input to the striatal MSNs by examining how much synaptic input is required to keep the cell in the depolarized state, as well as comparing the structure of the cortical input

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to the membrane potential of the MSN cell. We have also examined the questions of whether these cells are intrinsically bistable with or without DA modulation and how DA modulation affects the integration of inputs. With a large network model of MSNs and interneurons, we have begun to investigate the effect of realistic levels of lateral inhibition in the striatal network of MSNs.

Intrinsic Properties and Membrane Behavior of MSP Neurons

Among the most intriguing aspects of the MSP neurons are their intrinsic membrane properties and their behavior in response to synaptic input. These cells have a large dominant inwardly rectifying potassium current with a low reversal potential (–90 mV) that is open at rest [36] and leads to a very low resting membrane potential (∼–85 mV) [41,28]. When the MSP cells are depolarized, another set of potassium currents (KA-type) dominates the behavior of the membrane [40]. These potassium currents largely mediate two defining electrophysiological characteristics of the MSP cells – a depolarizing ramp preceding the first spike in response to current injection and a bimodal membrane potential in response to sustained synaptic input. The transitions between the hyperpolarized state (the "down" state) and the depolarized state (the "up" state) lead to the characteristic bimodality of the Vm under specific input conditions. This bimodality has also been termed "bistability" due to the apparent inability of the cell to remain in between these two states under anesthesia and in vitro [15,37].

Anesthetics tend to synchronize the afferents of the striatum and could therefore account for the changes in afferent input that lead to these state transitions [5,8,23]. Recently, an awake head-fixed intracellular rat preparation was utilized to demonstrate that MSP cells' Vm rarely enters the "down" state in the awake animal, but that under slow-wave sleep conditions the Vm becomes bimodal as in the anesthetized condition [22]. Despite the fact that transitions to the "down" state may not occur in the awake animal, much has been inferred from the transition of these cells between states. Many models of how the basal ganglia function are dependent on linearity of the MSN membrane response and how fast inputs are integrated at any given time.

Computational Model

In order to address how the MSP cell integrates inputs under various conditions, we constructed a computational model of the MSP neuron [42]. The 189-compartment Nacb MSP cell was developed using the NEURON simulation environment [18] and includes most of the known ionic currents in these cells.

The model is the first MSP model to include all reported species of calcium and calcium-dependent potassium currents as well as recently updated parameters for several potassium currents. Whenever feasible, channels were taken directly from published reports in striatum and utilized in the model with minimal adjustments (for details see [24,42]). The cell was tuned solely by balancing the maximum conductance levels of intrinsic currents against each other to match in vitro adult rat MSP current injection data. To test theories predicting DA modulation of excitability and bistability of the MSNs, we attempted to maximize the number and concentration of calcium channels in the model, while matching the current injection data as closely as possible [24]. Calcium channels are heavily modulated by DA and have therefore been predicted by many to be one of the major ways DA can modulate the intrinsic activity of the MSP cells [27,31]. The model receives synaptic input from simulated spike trains via NMDA, AMPA, and GABA-A receptors, and the frequency of these inputs is modulated to generate hyperpolarized or depolarized states in the cell.

Functional role of MSP Cells and DA Modulation

The behavior of striatal MSP cells under DA modulation has been thoroughly investigated under many different conditions, often with conflicting results in terms of cell output and membrane excitability (for review, see [24]). Further complicating the description of the response to DA modulation is the separation of MSP cells in the striatum into two types – those that express D1-like receptors (D1R) and those that express D2-like receptors (D2R). In the dorsal striatum, the outputs of these two populations are also segregated, with the D1R population projecting primarily to the internal globus pallidum (GPi) and the substantia nigra pars reticulata (SNr) and the D2R MSNs projecting to the external globus pallidum (GPe) [13]. Another aspect that makes the examination of these issues difficult is that there are two types of signaling associated with the DA neurons that project to the striatum, termed phasic and tonic [14]. Phasic DA cell firing has been proposed to be an error prediction signal about rewards, with theorists suggesting that this represents a signal that can be utilized for a type of temporal delay learning, while tonic DA release may serve to maintain the current levels of DA in the striatum [10,33].

Some models suggest that the modulation by dopamine (DA) of the currents that dominate state transitions is one of the primary ways that DA exerts its effects [15,25,27]. These models suggest that the hyperpolarized state might serve to filter out uncoordinated inputs, while the depolarized state would be more prone to fire to less coordinated input. In this manner the cell might switch from being a coincidence detector to an

integrator of relevant information. Several models of striatal/basal ganglia function have also been built on the idea that striatal MSNs are inherently bistable, some of which are discussed below.

Modulation Conditions

In order to test whether DA modulated these aspects of the behavior of our model MSN cell, we created four modulation conditions to study whole-cell D1- and D2-receptor-mediated modulation of the MSN: specifically (1) D1 modulation of intrinsic (sodium, potassium, and calcium channels) channels (D1 intrinsic condition), (2) D2 modulation of intrinsic channels (D2 intrinsic condition), (3) D1 modulation of both intrinsic and synaptic (AMPA and NMDA) channels (D1 All), and (4) D2 modulation of intrinsic and synaptic channels (D2 All). These modulation conditions were based on an extensive review of the dopaminergic modulation literature [24]. Each condition combines reported conductance changes and voltage shifts for

Table 15.1 Dopaminergic modulation conditions for MSN model. Values are percentages of the unmodulated conductance, i.e., 100% indicates no modulation. (From [24], used with permission)

Modulation conditions			
		D1 intrinsic	D1 All
D1	NaF	95%	95%
	h shift	0 mV	0 mV
	Ca P/Q	50%	50%
	Ca N	20%	20%
	Cav1.3	100%	100%
	m shift	–10 mV	–10 mV
	Cav1.2	200%	200%
	Kas	No change	No change
	KIR	125%	125%
	NMDA	100%	**130%**
	AMPA	100%	**100%**
		D2 intrinsic	D2 All
D2	NaF	110%	110%
	h shift	3 mV	3 mV
	Cav1.3	75%	75%
	m shift	0 mV	0 mV
	Cav1.2	100%	100%
	Kas	110%	110%
	KIR	100%	100%
	NMDA	100%	**100%**
	AMPA	100%	**80%**

individual channels in order to represent the expected net effect of exogenously applying dopamine to MSNs expressing solely D1 or D2 receptors (Table 15.1).

D1-Receptor-Mediated Modulation and Nonlinearity in the Model MSN

Our study addressed three different hypotheses regarding the function of DA modulation in the striatum. The first hypothesis suggested that D1 receptor (D1R) activation should make the MSN's response to synaptic input more nonlinear [15,17,27]. Specifically, this hypothesis proposes that D1R modulation increases the preference of an MSN to reside in one of two states – up (depolarized ~–60 mV) or down (hyperpolarized ~–85 mV). We tested this hypothesis primarily by examining the model's response to step changes in synaptic input applied at equally spaced increments from low to high frequency (Fig. 15.1A, inset). Each trace in Fig. 15.1A represents the response of the MSN to a different frequency of synaptic input (averaged across 18 trials); each dot in Fig. 15.1B represents the average value of the last 50 ms of the corresponding trace in Fig. 15.1A. Figure 15.1C is a histogram of the membrane potential distributions in Fig. 15.1A, over all trials, 670 ms after the frequency step. If D1R activation induced nonlinearity in the membrane's response, most of the traces in Fig. 15.1A would be near –85 or –55 mV, with a region in between in which there were few traces. Put another way, the curves in Fig. 15.1B would more closely resemble a sigmoid than a line – the cell would gravitate toward either the down state or the up state and transition quickly between them. Nonlinearity would be evident in the histograms in Fig. 15.1C as a clear distribution into two populations of potentials, one centered near –85 mV and one near –60 mV.

We did not observe nonlinearity in the unmodulated state, and neither D1 modulation of intrinsic properties (D1 intrinsic) nor D1 modulation of both intrinsic and synaptic properties (D1 All) visibly enhanced nonlinearity (Fig. 15.1A–C). Following a recent study describing NMDA-dependent bistability in an MSN-like two-compartment computational model [21], we also examined whether very high levels of NMDA might be able to induce nonlinearity or bistability (Fig. 15.1A–C, bottom). We found that an NMDA:AMPA ratio above 2.5:1 was required to induce two markedly distinct membrane potential states (Fig. 15.1C, bottom); this value appears to be outside the range of physiological ratios [26]. NMDA:AMPA ratios between 1:1 and 2.5:1 were not capable of inducing this behavior (data not shown). Taken together, this data suggests that D1 modulation does not enhance nonlinearity, except at potentially non-physiological levels of NMDA conductance.

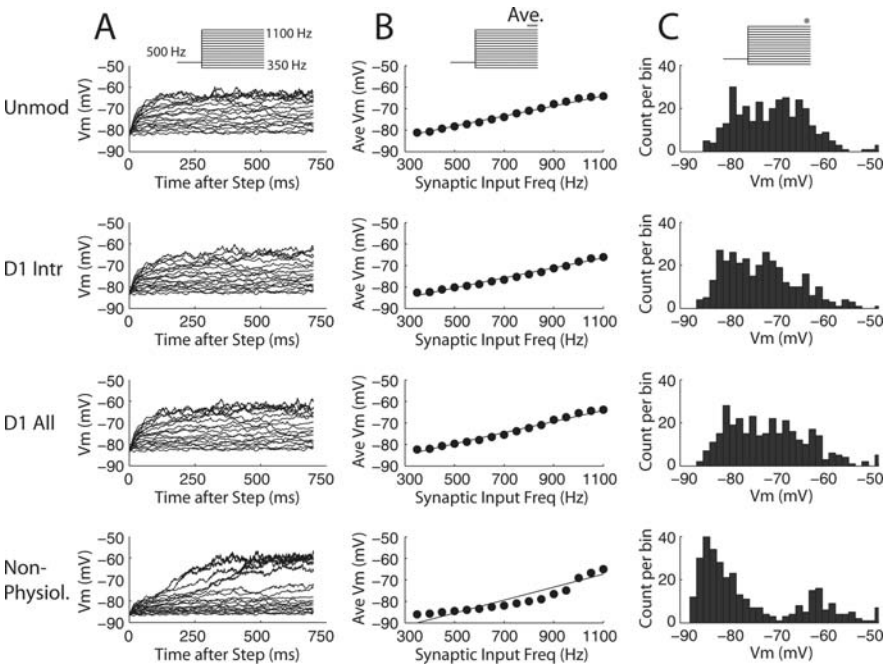

Fig. 15.1 Effects of D1R-mediated modulation on nonlinearity, hysteresis, and negative slope conductance in the model. (**A**) Averaged response (18 trials) of the model to constant frequency synaptic input in the unmodulated state (Unmod) and following D1R-mediated modulation of intrinsic channels (D1 Intr) and both intrinsic and synaptic channels (D1 All). In each trial, the model received synaptic input at 500 Hz for 200 ms; then the input was stepped to a new frequency and held for 700 ms (see inset). The input steps were linearly spaced between 350 and 1100 Hz. (**B**) Averaged membrane potential (18 trials) vs. synaptic input frequency. Each point represents the average of the last 50 ms of the corresponding trace in Fig. 15.3A, with a least squares linear fit drawn through the data for comparison. Neither the unmodulated, the D1 intrinsic, nor the D1 All states exhibited nonlinearity. Upon dramatically increasing the NMDA conductance (500% of baseline), the model exhibited some nonlinearity (non-physiol); however this may be a non-physiological level of NMDA. (**C**) Membrane potential distribution in all trials 670 ms after the step in synaptic input frequency in the unmodulated (Unmod) condition and following D1R modulation of intrinsic channels only (D1 Intr) and D1R modulation of intrinsic and synaptic channels (D1 All). The distribution was unimodal in these conditions, but upon dramatically increasing NMDA, the distribution became bimodal (non-physiol). (Modified from [24], used with permission)

Excitatory/Inhibitory Properties of DA Modulation

The second hypothesis that we examined suggested that DA regulates the excitability of MSNs in response to synaptic input. Specifically, according to the classical model of basal ganglia function [1,2], D1R activation should increase MSN activity in response to synaptic input, while D2R activation should

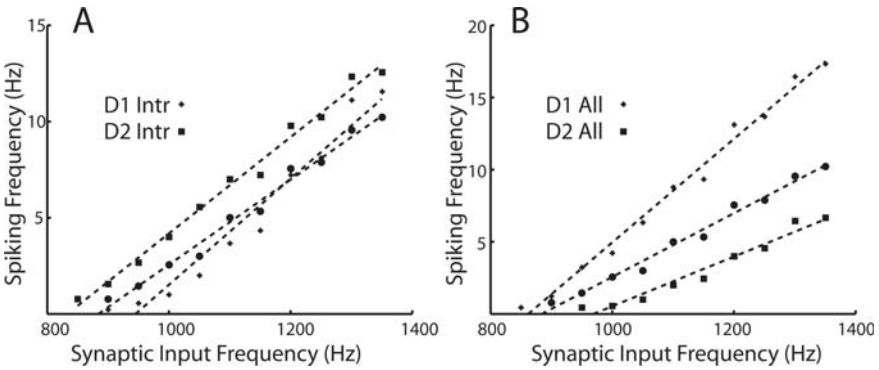

Fig. 15.2 (**A**) Spiking frequency vs. synaptic input frequency for unmodulated (*circles*), D1R (pluses – D1 Intr), and D2R (*squares* – D2 Intr) mediated modulation of intrinsic properties only. (**B**) D1R modulation of both intrinsic and synaptic properties (D1 All) leads to excitation at all input levels (pluses). D2R modulation of both intrinsic and synaptic properties (D2 All) leads to inhibition at all input levels (*squares*). The effects of synaptic modulations counteract the effects of intrinsic modulations on excitability in both the D1 All and D2 All conditions, and the resulting effects in these conditions agree with previous proposals regarding DA's effects on MSP neuron excitability. (Modified from [24], used with permission)

decrease MSN activity in response to synaptic input. We examined the effects of DA modulation on MSN response to synaptic input at different input frequencies (Fig. 15.2). We found that D1 intrinsic modulation changed the slope of the response of the cell to different synaptic input frequencies, i.e., the cell spiked more in response to higher synaptic input frequencies, but less in response to lower synaptic input frequencies, compared to the unmodulated cell (Fig. 15.2A). D2 intrinsic modulation increased the spike response of the cell to all synaptic input frequencies, though we found that this was almost entirely dependent on the modulation of the fast sodium current, which is not currently well described. Including synaptic modulations with the intrinsic modulations caused D1 modulation to become wholly excitatory, while preserving the gain change of the intrinsic modulations. Including D2 synaptic modulation reversed the excitable effects of the D2 intrinsic modulations and made the net effect wholly inhibitory, in accordance with the classical model of DA inhibition of D2-expressing cells.

Therefore, our two major conclusions regarding the net effects (intrinsic plus synaptic modulations) of DA regulation of MSN excitability are as follows: (1) D1 and D2 modulation affect MSN response to synaptic input in a manner consistent with the classical model of the basal ganglia and (2) the net effects of D1 and D2 modulation are primarily, if not entirely, determined by DA's effects on synaptic currents. Stated another way, intrinsic and synaptic modulations do not interact cooperatively to regulate MSN excitability. Our interpretation of this lack of synergy is that while DA may regulate MSN excitability in keeping

with the classical BG model, it does not appear to be the primary or sole purpose of DA modulation.

Dopamine and Temporal Integration Properties of the MSN

It has been suggested that one of the functions of the basal ganglia is to integrate information over a longer period of time during sequences and habit learning [3]. The MSP cell is in a unique position to integrate input from many areas of the cortex and thalamus at a given time. These inputs may be representing the "state" of the cortex, and this would be an ideal way for a training signal to modulate the current weights of the network involved in a given task. Our results suggest that the MSP cell integrates the previous 50 ms of afferent input and that the membrane potential at any given time is an extremely faithful representation of the inputs that the cell has received. In order to investigate whether DA modulation might change this integration time window of the MSN, we compared the model's somatic voltage to a sliding window average of the synaptic inputs to the model. By changing the size of the sliding window and calculating the correlation coefficient between the membrane voltage and the sliding window average, we could estimate over what temporal range the MSN was integrating inputs (Fig. 15.3). We found that DA does appear to

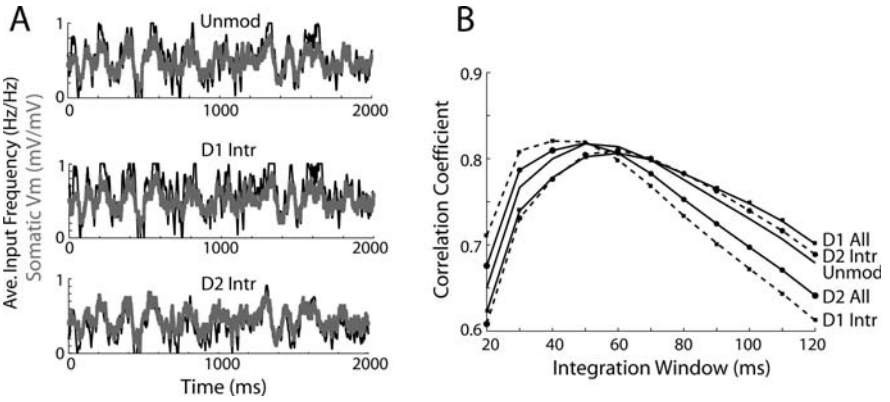

Fig. 15.3 Effects of D1R- and D2R-mediated modulation on MSN temporal integration of synaptic inputs. (**A**) Somatic Vm (*grey*) and averaged input frequency using a sliding window of 50 ms (*black*) in the unmodulated (*top*) state and following D1 intrinsic (*middle*) and D2 intrinsic (*bottom*) modulation. (**B**) Zeroth-lag correlation of somatic Vm and averaged input frequency using different sliding window sizes. The unmodulated model correlates best with a window size of 50 ms. D1 intrinsic modulation (*dashed squares*) decreases this window size to 40 ms, while D2 intrinsic modulation (*dashed circles*) increases it to 60 ms. Including synaptic modulations reverses this effect, with D1 All (*solid squares*) modulation increasing the window to 60 ms and D2 All (*solid circles*) modulation decreasing it to 50 ms. The correlation was calculated over 13 s of subthreshold synaptic input (800 Hz). (Modified from [24], used with permission)

affect the temporal integration window of the MSN with only intrinsic modulations, shifting the best correlation value between 40 and 60 ms. Interestingly, we again found that the effects of synaptic modulations for both D1 and D2 counteracted and overcame the effects of the intrinsic modulations, suggesting that while DA may regulate the temporal integration window of the MSN, this is not its primary purpose. These results suggest that the temporal window of integration is relatively stable even under DA modulation conditions and that synaptic plasticity or selective modulation of spines may be necessary to shift the importance of a given set of inputs, rather than temporarily decreasing the time window of integration for the inputs.

Local and Network Level Inhibition

An important feature of the striatal network is the local GABAergic inhibition that each MSP cell receives, both feed-forward input from interneurons and lateral inhibition from other MSP cells. In order to examine the differing roles of inhibition in the striatal microcircuit, we have developed a network of 1728 MSNs with 77 GABAergic fast-spiking feed-forward interneurons. MSNs utilized in the network model were identical to those used in the previous studies, with no reduction in channel descriptions or compartment number. MSNs were randomly connected to each other with a probability of 15.5%, with a maximum number of contacts of three per cell (Fig. 15.4A). FSIs were connected to 25% of the MSNs within 250 μm, with between 7 and 12 contacts from the IN to each MSN it contacts [35]. By feeding the same set of afferent inputs multiple times into the network we are able to examine the response of the network to these inputs under varying levels of lateral inhibition.

When the response of the network to the same inputs with and without lateral inhibition is compared, lateral inhibition reduced spiking in network significantly and shifted the frequency distribution of the output (Fig. 15.4D). When the membrane potential of an individual MSN in the network is examined, it is clear that not only have individual action potentials been eliminated, but that others have been shifted in time when lateral inhibition is turned on (Fig. 15.4E). This change in spike timing could in turn affect aspects of synaptic plasticity dependent on spike timing (STDP). Even very small weights (0.1, results not shown) of inhibitory connections between MSP cells may therefore be having a profound effect on information processing in the striatum. As mentioned in the previous chapter, DA modulation of these lateral inhibitory contacts may be an important aspect of network function, and also another aspect of striatal function that the loss of DA in PD may affect.

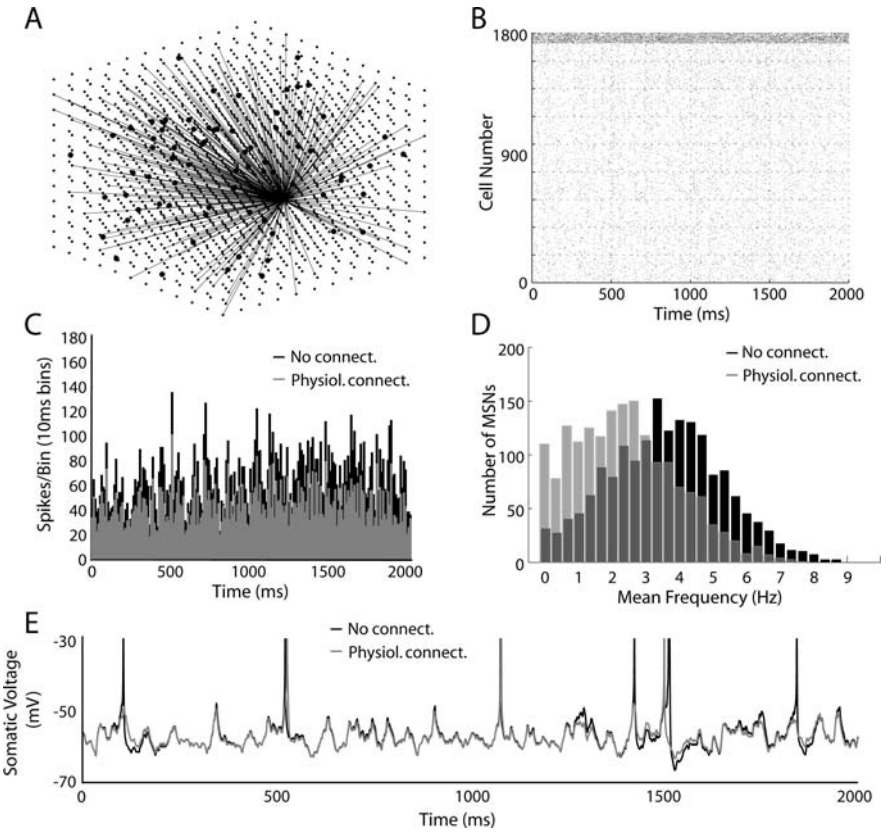

Fig. 15.4 Network model of striatal neurons. (**A**) Lateral connectivity of a single medium spiny neuron (MSN) in the network model. Small dots are MSNs, large dots are fast-spiking interneurons (FSI). (**B**) Raster plots showing the spiking activity of all 1728 MSNs (cell numbers 0–1728) and 77 FSIs (cell numbers 1729–1806) for the 2 s simulation. (**C**) Histogram showing spiking activity in 10 ms bins for all MSNs in the network model with no lateral connections (*black*) and physiological connections (*grey*). (**D**) Histogram showing number of MSNs spiking at a given frequency over the 2 s of simulation. Including physiological levels of lateral inhibition (*grey*) reduces the most common spiking frequency from 3.3 to 2.7 Hz. (**E**) Voltage trace of one MSN in the network. With exactly the same synaptic input for the whole network, lateral inhibition (*grey*) prevents several spikes and shifts the timing of others, compared to the trace where no lateral inhibition was present (*black*)

Implications of MSN Responses for BG Models

Our results demonstrate how MSN cells integrate their afferent input and the DA modulation of this integration. Our findings provide support for certain larger network models of BG function as opposed to others. While many models of the BG and corticostriatal interactions rely on functional units that are bistable [15,21], our results suggest that these cells are not bistable even

under extreme DA modulation conditions of intrinsic and synaptic currents throughout the cell. Another set of models uses linear responses to inputs in the MSNs, which are more in accordance with our results [20]. Certain other models describe the feature detection and potential dimensionality reduction of the inputs to the striatum and are therefore well supported by our results [3,19]. Indeed, the best description of the MSN's response to afferent input appears to be feature detection, as the representation of the cortical afferents features appears to be faithfully represented by the membrane response. Which of these features are translated into spiking activity, and the relationship between these features and STDP, remains to be further explored, although a preliminary hypothesis has been put forth recently [24] as has a description of how LTP and LTD are regulated in the striatum via STDP [29].

Implications for Parkinson's Disease

The underlying etiology of PD is a loss of DA cells in the SNr that project to the dorsal striatum, which in turn leads to a loss of DA in the striatum. We found that DA acting at D1 receptors had a net excitatory effect on MSNs, while DA action at D2 receptors had a net inhibitory effect on MSNs. This agrees with the classic Albin–DeLong model and implies that the loss of DA in PD would decrease activity overall in the direct, D1R movement-facilitatory pathway, while increasing activity in the D2R pathway.

Following dopamine depletion in animal models of PD, glutamatergic synapses appear to undergo changes, specifically in the NMDA receptor, and the NMDA/AMPA ratio has also been demonstrated to increase on D1 (but not D2) neurons post-DA depletion [7,30]. Our modeling studies focused on the entrainment of MSNs to afferent oscillatory inputs suggest that there is a change in how the cell processes inputs when there is a change in the NMDA/AMPA ratio [42]. We predict from these previous studies that the changes in the NMDA/AMPA ratio will have profound implications for the cell's response to afferent input. The change in the NMDA/AMPA ratio will have an effect on how the cell responds to any oscillatory input, as it effectively shifts the time constant of the sum of the glutamatergic synaptic currents [42]. As well as the entrainment of cells to afferent oscillations (theta, beta, and high-voltage spindles), disruption of these time constants may affect interactions with locally mediated oscillations as well [9,4,12]. Indeed, NMDA antagonists, which could reduce the NMDA/AMPA ratio, have been suggested as an important group of potential treatments for PD [16].

The loss of DA in the striatum also leads to changes in the MSN cell structure and synaptic inputs [30,34,43]. Spine loss was recently demonstrated in animal models of PD, and the D2-receptor-expressing MSNs appeared to be selectively vulnerable to this loss [11]. This was surprising, since these are the same cells that are predicted to have an increase in output in the Albin–Delong model

during a loss of DA. However, there may be compensatory mechanisms attempting to reverse the loss of input, which may in turn be leading to the aberrant firing that leads to the symptoms of Parkinson's disease [11]. A decrease in the number of inputs required to fire the cell may also lead to the inability of the cell to represent as many cortical states as were previously encoded, which could lead to a disruption in movement-related cell activation. We hope to continue to utilize the single cell and network models presented above to further investigate these ideas in order to deepen our understanding of how the loss of DA modulation of corticostriatal interactions leads to such devastating effects in this disease.

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Part IV
Neurobiology and Pathophysiology of Parkinson's Disease

Chapter 16
Pathogenesis of Oxidative Stress and the Destructive Cycle in the Substantia Nigra in Parkinson's Disease

Emilio Fernández-Espejo

Fundamental Aspects of Reactive Oxygen Species

The dangers imposed by O_2 arise from its electronic structure. It contains two unpaired electrons with parallel spin states, located on the second sheet (L), concretely on 2py and 2pz orbitals. Electrons tend to distribute according to a minimal repulsion, and two electrons with the same spin repel each other with more intensity than when their spins are antiparallel. For these reasons, if O_2 is in contact with a reductant molecule, electrons are transferred to O_2 one at a time: the first occupies the 2py orbital and the second occupies the 2pz orbital. The first step gives the superoxide ion ($O_2^{\cdot -}$), and the second step (linked to the transfer of $2H^+$) gives hydrogen peroxide (H_2O_2). Two more electrons are needed to reduce O_2 to two molecules of water (Fig. 16.1). Hence this univalent pathway usually needs intermediates, and those intermediates include strongly oxidant reactive oxygen species such as superoxide ions and hydroxyl radicals.

Can Cell Death in Substantia Nigra be Caused by Oxidative Damage?

The generation of free radicals and oxidant reactive oxygen species (ROS) is part of the normal cellular metabolism. The most important ROS are superoxide ions ($O_2^{\cdot -}$) that can be produced by the transfer of electrons to O_2 in the electronic chain. It is important to note that the first line of defense of the organism is to minimize the production of $O_2^{\cdot -}$. Thus O_2 is converted directly into water (without intermediate molecules) mostly due to the action of

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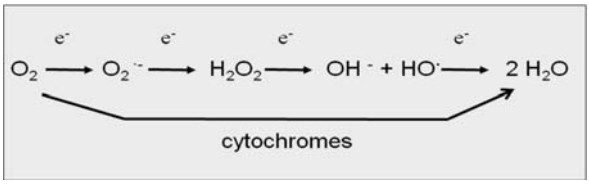

Fig. 16.1 Reduction of O_2 to water. Several oxidant intermediates emerge because electrons are transferred to O_2 one at a time. The first step gives the superoxide ion ($O_2^{\cdot -}$), and the second step (linked to the transfer of $2H^+$) gives hydrogen peroxide (H_2O_2). Two more electrons are needed to reduce O_2 to two molecules of water. In the cell interior, O_2 is converted directly into water (without intermediate molecules) mostly thanks to the action of cytochromes of the electronic chain, but around 0.1–1% of electrons transferred to O_2 can yield $O_2^{\cdot -}$

cytochromes of the electronic chain, but around 0.1–1% of electrons transferred to O_2 can yield $O_2^{\cdot -}$ (see Fig. 16.1). Free radicals are normally scavenged by cells, through conversion of superoxide ions to hydrogen peroxide (H_2O_2) under the control of superoxide dismutases (SODs), and then the formation of water from the reaction of hydrogen peroxide with reduced glutathione (Rd-GSH), under the control of glutathione peroxidase (GPx). The efficiency of the conversion of $O_2^{\cdot -}$ into H_2O_2 is unmatched, and several families of SODs have been imposed by evolution. For example, mammalian cells contain MnSOD in the mitochondria and CuZnSOD in the cytosol and other organelles. Overall, SOD is the first line of defense against $O_2^{\cdot -}$, and it is considered as the most effective antioxidant.

The H_2O_2 produced from $O_2^{\cdot -}$ is eliminated through catalases and peroxidases. The catalases carry out a dismutation reaction in which one H_2O_2 oxidizes another such that the one is converted to O_2 and the other to two molecules of water. Most catalases are heme-containing enzymes. Peroxidases use several reductants to reduce H_2O_2 to two molecules of $H_2O^{\cdot}$ (with the reactive species hydroxyl anion, OH^-, and hydroxyl radical, HO, as intermediate molecules), and the most important peroxidases in mammals are GSH peroxidases (GPx), those containing a prosthetic group with a selenocysteine residue. As discussed above, the group GSH reacts with hydrogen peroxide leading to the formation of water. GPx provides the second line of defense against hydroperoxides.

Oxidative stress results from insufficient scavenging of reactive oxidative species. Nigral dopaminergic neurons are exposed to higher oxidative stress because the metabolism of dopamine gives rises to dopamine–quinone species (SQs), superoxide radicals, and hydrogen peroxide, all oxidant molecules. Dopamine–quinone species are maintained at low levels through the action of glutathione, and excess formation of dopamine–quinone seems to be normally antagonized by reuptake of dopamine excess into lysosomes and endosomes by vesicular monoamine transporter (VMAT) action. These mechanisms are impaired in Parkinson's disease (PD). On the other hand, hydrogen peroxide is innocuous because it is rapidly degraded by GSH peroxidases, yet it can be converted into cytotoxic hydroxyl radicals, in a reaction catalyzed by iron

known as Fenton reaction. This reaction can be dangerously enhanced if GSH levels are reduced and there is an excess of iron (Fe(II)), as it seems to be the case in PD [1, 2]. The $OH^-$and $HO\cdot$ produced in the Fenton reaction are highly oxidant and can oxidize nucleic acids, proteins, and phospholipids. As mentioned above, superoxide is also a highly reactive molecule because of its conversion to hydrogen peroxide under the control of superoxide dismutase. However if nitric oxide levels are enhanced as it seems to be the case of PD, superoxide can react with nitric oxide to form peroxynitrite ($ONOO^-$) and hydroxyl radicals, strong oxidants [3]. NO and $O_2^{\cdot -}$ react at $-10^{10}M^{-1}s^{-1}$, and nitric oxide can also displace iron from ferritin thereby enhancing free iron levels [4]. These reactions could explain why SOD is enhanced in PD brains, likely as a homeostatic mechanism tending to reduce the formation of superoxide [5]. Figure 16.2 shows the main reactive oxidative species that contribute to the pathogenesis of PD.

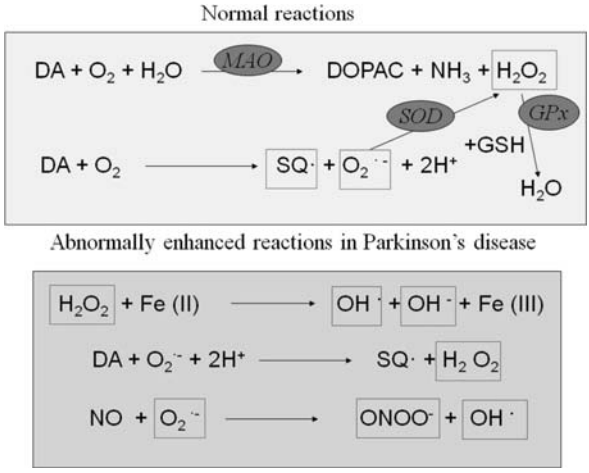

Fig. 16.2 Oxidative stress in substantia nigra neurons in Parkinson's disease. Normal metabolism of dopamine (DA) gives rise to its metabolite DOPAC and hydrogen peroxyde (H_2O_2) under the control of monoamine-oxidase (MAO), but also to oxidant species such as superoxide anions ($O_2^{\cdot -}$) and dopamine–quinones ($SQ\cdot$). Superoxide ions are converted into hydrogen peroxide (H_2O_2) under the control of superoxide dismutase (SOD), and then water is formed from the reaction of hydrogen peroxide with reduced glutathione (GSH), under the control of glutathione peroxidase (GPx). However, in Parkinson's disease, hydrogen peroxide can be converted into cytotoxic hydroxyl radicals, in a reaction catalyzed by iron (Fenton reaction). This reaction can be dangerously enhanced if GSH levels are reduced and there is an excess of Fe(II). The $OH^-$and $HO\cdot$ produced in the Fenton reaction are highly oxidant molecules. In the other hand, whether nitric oxide levels are enhanced as it seems to be the case in PD, superoxide can react with nitric oxide to form peroxynitrite ($ONOO^-$) and hydroxyl radicals, strong oxidants. DOPAC, 3,4-dihydroxyfenilacetic acid. Oxidant species are boxed

Substantia Nigra is Subjected to Oxidative Stress in PD: The Destructive Toxic Cycle

There are several indicators that the substantia nigra in PD is subjected to increased oxidative stress, and nigral iron levels have been found to be elevated in PD patients (129%) [1, 2]. Iron accumulation seems to occur as consequence of neuronal cell death since iron levels in the substantia nigra are not elevated in subjects with incidental Lewy body disease, not suffering from PD. However, accumulation of iron surely contributes to progression of cell loss. Levels of the antioxidant enzyme GSH are decreased by 40% in PD [6], although the role of this decrease remains to be determined since these levels are not considered to be dangerous. However, a strong decrease of GSH content has been detected in glia cells of nigral tissue of PD patients [7]. The cause of this decrease is not known, but clearly renders cells more sensitive to toxin action and potentiates the toxic effects of glial cell activation. Therefore, nitric oxide synthase can be enhanced through glial cell activation [8], leading to excessive production of nitric oxide and the oxidizing agents: peroxynitrite and hydroxyl radicals. Indeed, there is considerable evidence that oxidative stress in PD might result in part from the action of nitric oxide as revealed by increased immunoreactivity for inducible nitric oxide synthase in the substantia nigra of PD brains [8].

NO involvement in nigral cell death can also be related to the fact that NO can displaces iron from ferritin [4], and NO is known to inhibit complexes I and IV of the mitochondrial respiratory chain [9]. Indirect evidence implicates NO in neuronal degeneration because transgenic mice with a knockout of either neuronal NOS or inducible NOS are more resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that induces dopamine cell death in the substantia nigra [10, 11]. In fact, MPTP was discovered as a contaminant of illicit heroin, and it was found to cause PD within 7–14 days in heroin addicts [12]. MPTP is currently used to elicit dopamine depletion and to induce experimental parkinsonism in mice and non-human primates, remarkably similar to that observed in humans.

The excessive formation of reactive oxygen and nitrogen species in PD increases oxidative damage to proteins, lipids, and DNA. The levels of protein carbonyls, markers of protein oxidation, are twofold higher in the substantia nigra of PD patients [13]. The levels of lipid hydroperoxides, markers of oxidized lipids, are tenfold higher in the substantia nigra of PD patients [14]. Lipid peroxidation also leads to the production of 4-hydroxynonenal (HNE), and levels of HNE are known to be increased in dopaminergic cells in the substantia nigra (presence in Lewy bodies), and in cerebrospinal fluid in PD [15–17]. This substance is highly reactive, and it can decreases GSH levels as well as inhibits complexes I and II of the mitochondrial respiratory chain [15–17]. HNE is known to induce apoptosis after activation of caspases-8, -9, and -3, and also participates in DNA fragmentation. Finally, 8-hydroxyguanosine, a marker of oxidized RNA and DNA, is also enhanced in PD [18]. Toxic effects of NO and

peroxynitrites also can involve damage to DNA leading to excess of 8-hydroxyguanosine. As mentioned, there is increased immunoreactivity for inducible nitric oxide synthase in PD, and nitric oxide levels are enhanced in the substantia nigra [8]. All these oxidant effects are due to the fact that when a radical oxidizes any stable organic compound the first product is another radical derived from the organic compound. This can lead to chain reaction in which one initiation event can lead to the oxidation of many molecules. The formation of lipid peroxyl radicals is an example of such a reaction, as illustrated in Fig. 16.3.

From an experimental point of view, the sensitivity of the nigrostriatal pathway to selective toxins also demonstrates its vulnerability to free radical attack. For example, 6-hydroxydopamine (6-OHDA) destroys dopaminergic neurons through free radical-mediated mechanisms, and MPTP-induced impairment of the mitochondrial respiratory chain enhances superoxide formation. Both toxins are used in many laboratories for inducing parkinsonism in rodents and non-human primates (animal models of PD)

The strong oxidative stress in the substantia nigra can lead to misfolding of several proteins such as α-synuclein and parkin. Misfolded α-synucleins (as abnormal beta-sheets instead of normal alfa-helices) tend to form protofibrils which would precipitate forming fibrils which in turn constitute the core of the Lewy body. Protofibrils has been shown to be neurotoxic [19], pointing to the fact that protofibrils rather than fibrils could be the deleterious species. It was proposed that Lewy bodies could be a protective mechanism, although it has recently been demonstrated that the higher the number of Lewy bodies the stronger the degree of clinical symptoms [20]. The loss of normal function of α-synuclein could alter the normal vesicle function, since this protein is an important regulator of synaptic vesicle cycle, leading to enhanced intracellular levels of dopamine which are thought to further enhance intracellular oxidative

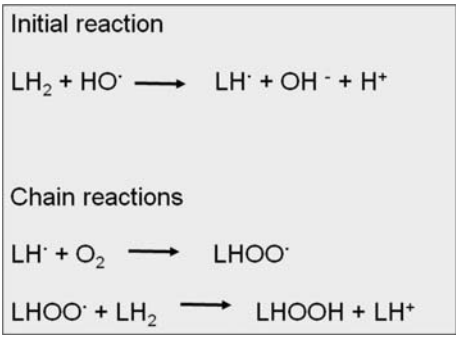

Fig. 16.3 Formation of formation of lipid peroxyl radicals in PD. Polyunsaturated lipids (LH_2) are oxidized by excess of HO· giving the highly oxidant hydroxyl anions. Later chain reactions give lipid peroxyl radical (LHOO·), and this chain reaction can amplify the consequences of the initiating event

stress [21]. Furthermore, accumulation of protofibrillar synuclein can saturate the ubiquitin–proteasome pathway (UPP).Normally proteins that are misfolded or damaged are eliminated by UPP [22], but there is evidence that the UPP is impaired in PD [23], not only due to accumulation of misfolded proteins but likely secondary to oxidative stress. A saturated or impaired UPP could lead to a defective handling of misfolding proteins, such as α-synuclein, and formation of Lewy bodies. Neurodegeneration could be accounted for these oxidative stress phenomena and UPP impairment, oxidation being the central factor (of unknown origin) leading to dopamine cell death in the substantia nigra. However other pathogenic mechanisms have also been proposed to be involved in neuronal destruction such as mithocondrial dysfunction, excitotoxicity, and inflammation, all of them related to each other and to oxidative processes, encompassing the so-called destructive toxic cycle. In fact, oxidative stress is intimately linked to mitochondrial impairment, excitotoxicity, and inflammation, and it is well known that the toxic effects of nitric oxide are also mediated through oxidative stress.

Mitochondrial Dyisfunction and the "Toxic Cycle"

Mitochondrial activity is decreased in the substantia nigra of PD patients [6], an effect that seems to be specific for PD. However, this decline is moderate and cannot account for the extensive cell death observed in the substantia nigra. There is evidence indicating that the damage does occur at the level of the mitochondrial DNA, which presumably might result in impairment of the respiratory chain leading to inhibition of complex I [24]. Iron-mediated free radical production can also inhibit complex I activity [25], and it is likely that there is an interaction between oxidative stress and mitochondrial inhibition, enhancing each other in a cycle of toxicity (Fig. 16.4). Mitochondrial electron transport is a major source of free radical production, and experimental impairment of complex I by MPP+ leads to excess formation of superoxide radicals [26], which in turn could further inhibit complex I [25, 27]. This "chicken and egg" situation has so far proved impossible to resolve, and it may be that these processes are so closely linked that it will not be feasible to determine which occurs first [28].

The mitochondria could also be target of quinone damage and dopamine metabolism that gives rises to excess dopamine–quinone species in PD, as already mentioned. Dopamine–quinones are highly oxidant molecules that could contribute to the strong oxidate process found in the substantia nigra of PD. On the other hand, dopamine–quinones can inhibit mitochondria NAOH reductase activity [29], and mitochondrial toxicity mediated by quinones has been suggested to produce apoptosis in PD [30]. Furthermore dopamine induces apoptosis in PC12 cells and other culture systems [31],

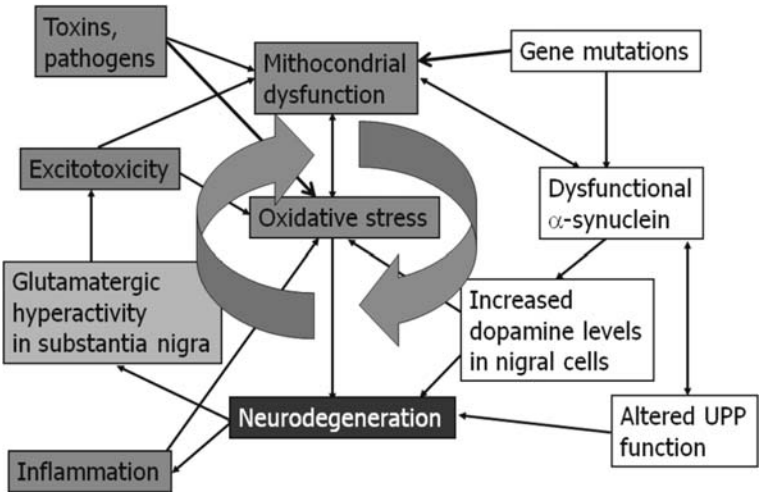

Fig. 16.4 The destructive toxic cycle. Oxidative stress, mitochondrial dysfunction, excitotoxicity, and inflammation enhance each other in a cycle of toxicity, leading to nigral cell degeneration in Parkinson's disease. Unknown toxins and/or pathogens as well as gene mutations can trigger the onset of the toxic cycle. Nigral dopamine neurons contain Lewy bodies (misfolded α-synucleins form fibrils which constitute the main component of the Lewy body), and the ubiquitin–proteasome pathway (UPP) is altered

particularly in presence of Fe(II) [32]. However, the role of apoptosis in PD remains controversial.

Excitotoxic Damage: Role of Glutamate

The notion of excitotoxicity was introduced by Olney to describe the neurotoxicity associated with administration of high concentration of exogenous glutamate or compounds acting as agonists of glutamate receptors [33]. In 1984, discoveries in the field of cerebral ischemia have encouraged the extrapolation of exogenous excitotoxicity to endogenous glutamate-mediated excitotoxicity [34]. Enhanced exocytosis of glutamate, and/or its deficient uptake, remains possible excitotoxic abnormalities. However, it seems that these effects take place at the synaptic level, and calcium influx would be linked to cell damage through increased permeability of AMPA–receptor–ionophore complexes to Ca^{2+} and abnormal sensitivity to NMDA receptors [35].

In PD, severe chronic dopamine depletion can result in subthalamic nucleus hyperactivity (see Fig. 1.1). This sustained increase activity in the subthalamic nucleus could facilitate glutamatergic release in the substantia nigra and elicit damage of dopaminergic cells by enhancing intracellular

calcium through the NMDA receptors. Overall, these changes could further aggravate the progression of the disease leading to accelerated nigral neurodegeneration.

Neuroinflammatory Phenomena in the Substantia Nigra

Despite the growing evidence indicative of a mitochondrial dysfunction and excitotoxicity in PD, there are also evidence pointing toward a neuroinflammatory mechanism for PD. For example, marked increase in cytokine levels have been found in the striatum and cerebrospinal fluid (CSF) of PD patients, including pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 mostly released by glial cells [36]. Upregulation of inflammatory-associated factors such as cyclooxygenase-2 (COX-2) and iNOS or inducible nitric oxide synthase have also been associated with PD [37]. There are also changes in neurotrophic factors, in both directions, such as increase of TGF-beta1, TGF-beta2, and TGF-alpha, together with a decrease in BDNF and NGF [38, 39]. Changes in trophic factors such as BNDF and NGF strongly suggest the presence of pro-apoptotic processes in the striatum of PD.

Experimental data indicate that pro-inflammatory cytokines are produced in the vicinity of dopaminergic neurons and therefore may be implicated in the pathogenesis of PD [40, 41]. The origin of pro-inflammatory cytokines in the PD brain seems to be derived from activated microglia. Major histocompatibility complex (MHC) molecules are also upregulated, as reported by McGeer and associates [42]. MHC plays a central role in antigen presentation to T cells, thereby initiating the immune response. It remains to be determined whether such upregulated expression of MHC has functional relevance. It has been postulated that, once the neurodegeneration process begins, T-lymphocytes are somehow activated within the substantia nigra, and they in turn induce the activation of glial cells. These glial cells would release pro-inflammatory factors and nitric oxide leading to apoptosis and oxidative stress, respectively [40]. Activation of the immune system and neuroinflammation could also be related to an immune response against aberrant products of dopamine oxidation. Thus, some PD patients would exhibit a specific response to dopamine-o-quinone-modified proteins or quinoproteins [43]. These data suggest that covalent modification of proteins due to dopamine oxidation could provide a specific antigenic determinant.

Neuronal death in PD can also be related to the induction of mitosis, as revealed by Höglinger and associates [44] who show that, in the postmortem human tissue, dopaminergic neurons aberrantly express mitosis-associated proteins, including the E2F-1 transcription factor, and appear to duplicate their nuclear DNA [44]. Hence, neuroinflammation would be another amplifying system leading to cell degeneration.

Conclusions

In summary, oxidative stress can initiate the driving force for neurodegeneration, a process that includes a destructive "toxic cycle" characterized by mitochondrial dysfunction, excitotoxicity, and inflammation, which in turn aggravate the degree of neurodegeneration. This latter would explain the rapid progression of PD once disabilities and clinical symptoms emerge, yet the mechanisms that trigger the oxidative stress remains to be determined [45].

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Chapter 17
Regulation of G-Protein-Coupled Receptor (GPCR) Trafficking in the Striatum in Parkinson's Disease

Marie-Laure Martin-Negrier, Céline Guigoni, Bertrand Bloch, and Erwan Bézard

Abbreviations GABA: gamma-aminobutyric acid, GPCR: G-protein-coupled receptor, GRK: G-protein-linked receptor kinase, MAPK: mitogen-activated protein kinase, D1R: dopamine receptor 1, DR5: dopamine receptor 5, D2R: dopamine receptor 2, D3R: dopamine receptor 3, D4R: dopamine receptor 4, cAMP: adenosine 3',5'-cyclic monophosphate, PD: Parkinson's disease, 6-OH-DA: 6-hydroxydopamine, LID: levodopa-induced dyskinesia, MPTP:1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, DA: dopamine, NMDAR: *N*-methyl-D-aspartate receptor, NR1: NMDA receptor subunit 1, NR2A: NMDA receptor subunit 2A, NR2B: NMDA receptor subunit 2B, DARPP32: dopamine- and cAMP-regulated phosphoprotein 32 kDa, PSD95: postsynaptic density protein-95, PSD: postsynaptic density

The striatum is a key element of basal ganglia involved in motor activity and cognitive function [1]. Dysregulation of its activity and/or physiology is involved in neurodegenerative diseases such as Parkinson's and Huntington's diseases and behavioural abnormalities such as addiction. Striatum is under the control of cortical, thalamic and nigral inputs and locally released neurotransmitter, i.e. GABA, acetylcholine, substance P enkephalins [2, 3]. Among many others, two key parameters that control neuronal activity and responsiveness are the abundance of neurotransmitter in the local environment and the density and availability of the receptors at the plasma membrane. The localization and the density of plasma membrane receptors are controlled by two metabolic pathways: the biosynthesis pathway which includes receptor sorting and targeting to the different neuronal membrane compartment and the endocytic pathway which includes endocytosis, receptor recycling and degradation [4–6].

A large body of literature has studied G-protein-coupled receptors (GPCR) trafficking in vitro and in vivo [7, 8, 4, 9, 10, 4, 11, 12, 6, 13–18]. Parameters that

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condition receptor trafficking include the nature of the receptors, the type of neurons, the neuronal compartment where the receptor is expressed and the local environment with regard to the nature of the neurotransmitter, its concentration and the type of stimulation, i.e. acute versus chronic stimulation [19–24].

In physiological conditions GPCR stimulation is rapidly followed by the so-called "homologous desensitization". First, agonist-activated GPCR is phosphorylated by G-protein-coupled receptor kinase (GRK), which is recruited to the plasma membrane. A beta arrestin then binds to the phosphorylated receptors and uncouples them from G protein resulting in the termination of GPCR signalling [25, 26]. Beta arrestin also promotes phosphorylated receptor internalization by recruiting endocytic protein complex [27]. More recent studies indicate that beta arrestin also promotes signal transduction by GPCR independently of G protein activation via, for instance, the mitogen-associated protein kinase (MAPK) cascades [28, 29].

Dopamine Receptor Trafficking Under Homologous Stimulation in the Striatum

Dopamine is a crucial modulator of the processing of cortical and thalamic striatal inputs carried by glutamatergic synapses on medium spiny neurons, which express glutamatergic and dopaminergic receptors. Two classes of GPCRs mediate dopaminergic transmission [30]. D1 class receptor that includes D1R and D5R is coupled to Gαs and enhance the production of the second messenger cAMP. D2 class receptor that includes D2R, D3R and D4R is coupled to Gαi/o and decreases the production of cAMP. D1R is expressed in the striatonigral medium spiny neurons constituting the direct pathway, whereas D2R is expressed in the striatopallidal pathway or indirect pathway [31].

D1R Trafficking

Under physiological conditions, D1R is mainly located extrasynaptically at the plasma membrane of cell bodies and dendrites of medium spiny neurons notably in spines at the edge of asymmetrical glutamatergic synapse [32, 33]. Very few receptors are located in the cytoplasm. Acute dopaminergic stimulation provokes dramatic modification in the D1R distribution [15]. It consists in a translocation of the receptor from the plasma membrane to cytoplasmic organelles, mainly the endosome compartment leading to a dramatic decrease of plasma membrane density of the receptors. These changes are reversible and this recovery is mediated by receptor recycling. Whatever the nature of the stimulation is, i.e. specific D1R stimulation with D1R agonist or stimulation of

all dopamine receptor subtypes with endogenous dopamine, the D1R behaviour is the same [15].

Chronic dopaminergic stimulation, as in dopamine transporter knock-out mice, induces a radical different pattern of D1R redistribution regarding acute stimulation. Indeed, an exaggerated accumulation of the receptor in the cytoplasmic compartments involved in synthesis, i.e. the rough endoplasmic reticulum and Golgi apparatus and great lowering at the plasma membrane in dendrites and cell bodies, is observed in these animals [20]. The decrease of the hyperdopaminergic tone leads to recruitment of the D1R to the plasma membrane of cell bodies with concomitant decrease in the endoplasmic reticulum [20].

Striatal D2R Trafficking

In physiological condition D2R is mostly expressed in striatopallidal medium spiny neurons [34, 31]. At odds with D1R, it is expressed mainly in the cytoplasm of cell bodies and equally distributed between the plasma membrane and the cytoplasm in dendritic spines [35–37]. Data obtained in vivo and in vitro indicate that under acute stimulation, membrane-bound D2R is endocytosed and efficiently recycled back to the membrane [16, 38].

Dopamine Receptor Trafficking in Parkinson's Disease

Parkinson's disease (PD) is characterized by a massive loss of nigral dopamine neurons that results in a dramatic reduction of dopamine concentration in the striatum. Experiments carried out on models of PD indicate that dopamine depletion induces an increase in D1R density at the plasma membrane with a decrease in intracytoplasmic receptors suggesting receptor recruitment to the plasma membrane from the cytoplasmic pool [20, 37]. In contrast, D2R distribution does not exhibit significant differences compared to physiological conditions [37]. Dopamine depletion induces an increase of D2R binding without change for D1R binding [39]. In vitro, D1R stimulation in striatal cultures deprived with dopamine leads to internalization of the receptor [40]. Analysis at ultrastructural level in PD patients with unclear pharmacological status at the time of death suggests an increase of D1R in the cytoplasm of medium-sized neurons [41]. In the same way, rats with a unilateral 6-hydroxydopamine (6-OH-DA) nigrostriatal lesion and treated with a D1R agonist show the same D1R localization suggesting that this internalization is the result of the L-dopa therapy [41].

In L-dopa-induced dyskinesia (LID), which represents troublesome side-effects of L-dopa therapy for PD, a few reports are available concerning dopaminergic receptor trafficking. First, the total D1R immunoreactivity is increased in accordance with increased dopamine receptor signalling [37, 39]. At electronic level quantitative analysis indicates an increase of the overall expression without

modification of the relative number of receptor at the plasma membrane versus cytoplasmic compartment compared to physiological condition [37]. These results suggest an impairment of the endocytosis pathway in dyskinetic condition as these data have been obtained in animals killed at the peak of L-dopa plasmatic concentration, and therefore behavioural effect, which normally should provoke a massive internalization of the receptor.

To understand this impairment of dopamine receptor trafficking, molecules mediating GPCR trafficking, especially GRKs and arrestins have been studied. Arrestins 2 and 3 are ubiquitous in the brain and five of seven GRKs (GRKs 2, 3, 4, 5, 6) are expressed in the brain [42–46]. Western blot experiments have shown that MPTP lesion induces an increase of the expression of the sole arrestin 2 in the striatum and among GRKs which globally are also affected, GRK6 presents the greatest variation with an increase of its expression [29, 39]. L-Dopa therapy reverses all these modifications with a normalization of their expression [39, 29]. In dyskinetic monkey, only an increase of GRK3 in a part of the striatum (ventral striatum) was detected [29]. Results obtained with the same procedure in rat model of PD (6-OH-DA-lesioned rat) display some discrepancy. In dopamine-depleted striatum the expression of arrestins 2 and 3 is modified after 6-OH-DA treatment and after L-dopa therapy. GRKs expression presents modification with notably as in the MPTP model an increase of the expression of GRK6 which in rat is reversed by pergolide treatment but not L-dopa therapy [47].

Altogether, these data indicate that dopamine depletion induces an impairment of the expression of arrestins and GRKs especially arrestins 2 and GRK6 and that these modifications are reversed by dopa therapy. However these modifications appear not compensatory to dopamine depletion but rather a complex change in signalling mechanisms induced by dopamine depletion and treatment. Indeed as D1R recruitment at the membrane compensates the DA depletion, a downregulation of arrestins and GRKs was expected rather than the increase observed [29]. It appears also that LID is not linked to an abnormal regulation of these proteins as no difference of arrestins and GRKs expression is observed compared to non-dyskinetic animals. However a possible relative deficiency of these proteins compared to the dramatic increase of D1R expression could explain the impairment of D1R internalization in LID. All these studies have been performed without studying the subcellular compartmentalization and trafficking of arrestins and GRKs which may be a major parameter to understand their activity and their influence on dopamine receptor trafficking in pathological conditions. In this respect, subcellular fractionation studies showed that each of these molecules has distinctive subcellular distribution and that modification induced by DA depletion and L-dopa therapy concerns specific subcellular fractions [47]. For example, the concentration of arrestin 3 in total cell lysate of dopamine-depleted striatum is unchanged after L-dopa therapy, whereas an increase in the membrane fractions is observed [47]. However the consequence of these modifications on dopamine receptor trafficking remains difficult to understand regarding the lack of information concerning the specific role of arrestins and GRKs isoforms on GPCRs.

Glutamate and Dopamine Receptor Trafficking Under Heterologous Stimulation

Even though in PD the first abnormality is the loss of dopaminergic neurons, it leads secondary to alteration in glutamate receptor trafficking, especially NMDA receptor class (NMDAR). Morphological analyses have demonstrated the close proximity of D1R and NMDAR in the dendritic spines of medium spiny neurons of the striatal direct pathway [48]. Glutamatergic synapses are localized on the head of the spines in which the glutamatergic effect is mediated by NMDAR and non-NMDA glutamate receptors, whereas dopaminergic input terminates on their shaft and stimulates D1R. In the adult striatum, NR1, NR2A, NR2B are the predominant subunits expressed and assembly to constitute tetrameric complexes of NR1 and NR2 [49, 50]. Biochemical and subcellular fractionation studies have shown that agonist D1R and not D2R stimulation induces an enrichment of NR1, NR2A, NR2B in the plasma membrane of the postsynaptic density and that this translocation is dependent upon tyrosine phosphorylation of NR2A and NR2B. Moreover this phosphorylation is dependent upon Fyn protein tyrosine kinase rather than DARPP-32 [50, 51]. In vitro trafficking studies of striatal neurons suggested that D1R stimulation induces a translocation of NMDAR from the cell body to the dendrites. In addition, this stimulation enhances the surface expression of NR1 and NR2B and the clustering with PSD 95 [52]. This enhancement of surface expression is associated with a tyrosine phosphorylation of the carboxy tail of NR2B by Fyn tyrosine kinase suggesting that the activation D1R increases the membrane density of NMDAR through activation of the protein kinase Fyn [52]. The mechanism that leads to this increase of surface expression of NMDAR needs further investigations to distinguish if D1R stimulation would induce an enhancement of NMDAR insertion rather than a blockade of receptor endocytosis or degradation. In animal models of PD, dopamine depletion results in a reduction of the number of NMDAR subunits in the postsynaptic density (PSD), which is reversed by L-dopa therapy. Indeed, a decrease of NR2B receptors is always found in striatal membranes without change for NR2A and some discrepancy for the NR1 subunit. These modifications are reversed by L-dopa therapy. In dyskinetic rats, it has been described complex redistribution of NMDAR with an enrichment of NR2A in the PSD and a decrease of NR2B that translocates to extrasynaptic sites [53]. These modifications are paralleled by modification of the distribution of scaffolding proteins of the PSD which participate among others to the stabilization of glutamate receptors at the synapse [54]. Indeed the decrease of NR2B in PSD is superimposed by the decrease of SAP 102 which is preferentially associated with NR2B [53]. In addition, the induction of dyskinetic motor behaviour in non-dyskinetic-treated animals by disrupting interaction of NR2B with scaffolding proteins outlined the critical role of these interactions in the subcellular localization of the receptor [53]. In a primate model of PD, the same decrease of

NR2B subunits reversed by L-dopa therapy is observed and a striking enhancement of NR2A subunits density in dyskinesia is observed [55]. In these models the phosphorylation status of NMDAR has been extensively studied showing some differences. Many groups have reported that dopamine depletion induces a decrease of NR1 serine and NR2B tyrosine phosphorylation and L-dopa therapy produced a hyperphosphorylation of NR1, NR2A and NR2B subunits in rats, whereas some other did not observed modification of the phosphorylation of the receptor in the same model animal or in primate model [53–56]. Altogether, these data outline the critical role of NMDAR trafficking perturbation in dyskinesia. Reciprocally D1R trafficking is controlled by NMDAR stimulation. In vitro, the activation of NMDAR induces a recruitment of D1R to the plasma membrane [57] and trap diffusible D1R to dendritic spines [58]. In addition, direct physical interaction between D1R and NMDAR has been reported. It has been shown that D1R interacts with both NR1 and NR2A [59–61] and that in 6-OH-DA-lesioned rats, D1R/NMDAR complex decreases in postsynaptic density [61]. Even if the mechanism of these interactions remains unclear, the efficiency of NMDAR antagonist to decrease dyskinesia outlined the importance of these interactions.

Conclusions

Our recent work has clearly demonstrated that the DA-depleted PD-like situation is characterized by (i) an overrepresentation of D1R at the membrane level and (ii) an increased signalling activity of both the canonical (DARPP-32 mediated) and non-canonical (Ras-ERK-mediated) signalling pathways. Such hyperactivity is seen also in LID as is the unexpected over-presence at the membrane of D1R. Overall, our data suggest that both the homologous and heterologous desensitization processes are impaired in LID as, in relative values, all parameters are down-regulated in LID versus the control or MPTP situations. The machinery exists but is, relative to D1R for instance, down-regulated. LID would therefore result from the impossibility of D1R to desensitize and internalize (or to an increased turn-over). Precise mechanisms responsible for this dramatic effect are now under investigation in our lab and should soon lead to therapeutic interventions.

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Chapter 18
Atypical Parkinsonism in the French West Indies: The Plant Toxin Annonacin as a Potential Etiological Factor

Annie Lannuzel and Patrick Pierre Michel

This chapter describes the phenotypic and clinical features of an atypical parkinsonian syndrome endemic to the Caribbean island of Guadeloupe. The clinical entity was much more frequent than idiopathic Parkinson's disease. It corresponded to a unique combination of levodopa-resistant parkinsonism, tremor, myoclonus, hallucinations, REM sleep behavior disorder and fronto-subcortical dementia. Epidemiological and experimental studies suggested that the neurological syndrome resulted from chronic intoxication by a mitochondrial complex I inhibitor, the plant toxin annonacin.

On the French West Indian island of Guadeloupe, atypical parkinsonian patients represent two-thirds of all cases of parkinsonism, which is exceptionally frequent compared to epidemiological data from European countries where atypical parkinsonism accounts for only ~5% of all cases. The clinical entity was a unique combination of levodopa-resistant parkinsonism, tremor, myoclonus, hallucinations, REM sleep behavior disorder and fronto-subcortical dementia. Two subgroups of patients were distinguished on the basis of the presence or the absence of supranuclear gaze palsy. In patients with oculomotor signs that came to autopsy, neuronal loss was found to predominate in the substantia nigra and the striatum, but other brain areas were also affected, including the frontal cortex. In addition, tau-containing lesions were detected throughout the brain. Epidemiological data suggested a close association of the disease with the regular consumption of soursop, a tropical annonaceous plant. Experimental studies performed in midbrain cell cultures identified annonacin, a selective mitochondrial complex I inhibitor contained in fruits and leaves of soursop, as a probable etiological factor. Consistent with this view, chronic administration of annonacin to rats through Alzet osmotic minipumps showed that annonacin was able to reproduce the brain lesions characteristic of the human disease.

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Introduction

There is an abnormally high frequency of atypical parkinsonism in Guadeloupe [3], a French Caribbean island with 420,000 inhabitants. Only one-third of the patients had idiopathic Parkinson's disease (I-PD) as compared to 70% in European populations [10]. This high percentage of patients with atypical parkinsonism led us and others to conduct studies (1) to further characterize this syndrome at the clinical and neuropathological level, (2) to determine whether the abnormal distribution of PD-related syndromes in Guadeloupe had an environmental origin, (3) to identify the etiological factor(s) and (4) to explore the underlying pathophysiological mechanisms.

Clinical Features of the Disease Entity

Guadeloupean Atypical Parkinsonism Has Two Distinct Phenotypes

One hundred sixty-one patients with parkinsonism seen consecutively in the Neurology Department of the University Hospital at Pointe-à-Pitre, Guadeloupe, between September 2003 and September 2005, were thoroughly analyzed for their clinical, neuropsychological and radiological characteristics [18]. Patients with I-PD had unilateral onset, a good response to levodopa- and dopa-induced dyskinesias. Patients with atypical Caribbean parkinsonism developed levodopa-resistant motor symptoms, associated with early postural instability and dementia. Two different phenotypes were defined on the basis of the presence or the absence of progressive supranuclear palsy (PSP)-like oculomotor signs. The group of patients with oculomotor signs was referred as Gd-PSP and the other group as Gd-parkinsonism dementia complex (PDC) [18].

Aside from the oculomotor signs, the Gd-PSP patients differed markedly from patients with classical PSP. The major sign that differentiated Gd-PSP from classical PSP was the presence of hallucinations that were mainly visual and unrelated to medication; they were observed in 59% of the patients and in all three PSP-like patients that came to autopsy [4]. The Gd-PSP patients also differed from PSP patients by a higher occurrence of tremor (56 versus 17–37% [19]); in particular, a low-frequency and high-amplitude rest tremor was observed in 20% of Gd-PSP patients.

A large proportion of Gd-PSP patients (92%) were demented, compared with the 52–74% of PSP patients with dementia [23, 27]. A common pattern of cognitive dysfunction was found in both Gd-PSP and Gd-PDC patients [18]. Both Gd-PSP and Gd-PDC patients performed poorly on tests of executive functions involved in behavioral planning and adaptation. It should be noted that the severity of dementia was usually greater in the Guadeloupean patients than in classical PSP [18]. Dementia in Guadeloupean patients occurred within 3 years of onset of the

disease, excluding the diagnosis of I-PD with dementia. Although the presence of hallucinations was evocative of dementia with Lewy bodies [21], the patients did not have the fluctuation in cognition, attention and alertness characteristic of this disease. Fronto-temporal dementia (FTD) was also excluded since changes in behavior and personality characteristics of FTD [22] were not the first symptoms.

Neuroimaging Features of Atypical Parkinsonian Patients

The MRI scans were abnormal in almost all patients with atypical parkinsonism but were strictly normal in 50% of I-PD patients [18]. The neuroradiological profiles of the Gd-PSP and Gd-PDC patients largely overlapped. Widespread supratentorial atrophy and enlargement of the ventricles, particularly the third, were observed in both groups. Cortical atrophy was most pronounced in the frontal and temporal lobes in Gd-PSP patients and in the frontal and parietal lobes in Gd-PDC patients. Upper mesencephalic atrophy (hummingbird sign), considered as highly suggestive of PSP [26], was observed in 63% of Gd-PSP patients and was severe in half of them. Severe mesencephalic atrophy was significantly more frequent in Gd-PSP than in Gd-PDC patients. Mesencephalic atrophy in Gd-PSP was not correlated with disease duration. In conclusion, except for the greater severity of mesencephalic atrophy in Gd-PSP patients, the MRI patterns of Gd-PSP and Gd-PDC were alike.

Neuropathological Data

The pathological findings of patients who came to autopsy revealed a severe loss of dopaminergic (DA) neurons in the substantia nigra associated with intense gliosis. Non-DA neuronal populations were also affected in various brain areas, particularly the frontal cortex, the pallidum, sub-thalamic nucleus and pontine nuclei. Extensive accumulation of the microtubule-associated protein tau was observed in the brain of Gd-PSP patients, which is evocative of a tauopathy. Tau-containing lesions extended beyond midbrain areas to subcortical and cortical regions. At the cellular level, tau accumulated in neuronal and astrocytic processes (neuropil threads), whereas true neurofibrillary tangles were rare. Gel electrophoresis studies detected a major doublet of pathological tau, at 64 and 68 kDa, in brain tissue homogenates [4] which is compatible with the diagnosis of PSP [20].

REM Sleep Behavior Disorder in Patients with Guadeloupean Parkinsonism

Patients with Guadeloupean atypical parkinsonism and their spouses reported insomnia, dream enactment and violence during sleep [18], which is suggestive

of rapid eye movement (REM) sleep behavior disorder (RBD) [2]. This justified a study including sleep interviews, motor and cognitive tests and overnight video polysomnography in nine Gd-PSP patients [9]. RBD was found in 78% of Gd-PSP patients, symptomatic in all cases. In comparison, only 13% of patients with classical PSP developed symptomatic RBD [2]. The pattern of RBD in Gd-PSP, in which the latency and percentage of REM sleep were normal, differed from that observed in patients with classical PSP, who had delayed and shortened REM sleep.

Candidate Etiological Factors

Plant Toxins

The unusually high number of parkinsonian patients with atypical clinical features in Guadeloupe and the cross-ethnic representation of the patients suggested that an environmental toxin might be responsible for the cluster of cases. This hypothesis was supported by case–control studies [3, 18] showing that patients with atypical parkinsonism consumed significantly more fruit and infusions or decoctions of leaves from plants of the Annonaceae family, particularly *Annona muricata L.* (soursop, guanabana, graviola, corossol) than patients with I-PD or control subjects. The leaves of these plants are used in traditional Creole medicine from early childhood to old age, sometimes daily, for heart and digestive problems, for sedative purposes or to maintain general health. Comforting the idea that this family of plants played a key role in the development of the atypical parkinsonian syndrome in Guadeloupe, a similar clinical entity has also been associated to annonaceae consumption in patients of Caribbean origin living in London [7] and in PD patients from new Caledonia, a French Western Pacific island [1].

Other Potential Etiological Factors

The regular professional use of pesticides has been proposed to be a causative factor for PD, based on evidence from a number of epidemiological studies [11]. Some synthetic pesticides, in particular organochlorine derivatives [13] which have been widely used in Guadeloupe, might possibly be associated with the disease, but no epidemiological data are available as yet on the subject. Therefore, a possible role of pesticides in the etiology of Guadeloupean atypical parkinsonism cannot be totally ruled out. A genetic origin has also been proposed. However, less than 4% of atypical parkinsonian patients had family histories of parkinsonism whereas PD symptoms were found in 15% of the first- or second-degree relatives of I-PD patients [18], making a genetic origin of the disease unlikely.

The Complex I Inhibitor Annonacin as a Possible Etiological Factor

We first used cultures of midbrain DA neurons to determine which compounds contained in *A. muricata* might be responsible for the neurological syndrome. We showed that the two most abundant alkaloids in this plant, the benzyl-tetrahydroisoquinoline reticuline and the tetrahydroprotoberberine corexi-mine, were toxic to DA neurons [16]. The concentrations of the alkaloids causing DA cell death were, however, relatively high (ranging from 13 to 300 μM) and thus not likely to be reached in the brain of patients.

Neurodegenerative Changes Induced by Annonacin

This led us to focus our interest on another class of potentially toxic compounds, the acetogenins, in particular annonacin, for two reasons: (1) annonacin is present in the plant in large amounts [6]; (2) it is a very potent inhibitor of the mitochondrial respiratory chain, at the complex I level, a property shared by other parkinsonism-inducing compounds [8, 24]. A concentration of annonacin of 30 nM was sufficient to kill most of the DA neurons in midbrain cultures [17]. Interestingly, this concentration caused a 50% reduction of complex I activity but had no effect on complex IV. Annonacin toxicity involved ATP depletion, since (1) it was prevented when glycolysis was stimulated by high concentrations of glucose or mannose and, conversely, (2) was favored by the non-metabolizable glucose analogue deoxyglucose [17]. Of interest, annonacin was almost 1,000 times more toxic to cultured neurons than the benzyl-tetrahydroisoquinoline reticuline, the most abundant alkaloid in *A. muricata*, and approximately 100 times more potent than the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+), a complex I inhibitor that causes parkinsonism in humans and in animal models of PD after bioconversion of its prodrug MPTP [15]. Unlike MPP^+, annonacin was not a substrate of the dopamine transporter, which explains why its toxicity was not restricted to DA neurons. Nevertheless, this result fits with data showing that atypical parkinsonian patients develop severe neurodegenerative changes outside of the nigrostriatal pathway, in particular within the striatum and the cortex [4].

When administered systemically to rats via Alzet osmotic minipumps, anno-nacin entered the brain parenchyma, where it was readily detectable by mass spectrometry [5]. The lipophilic nature of annonacin which facilitated its incor-poration and retention in brain parenchyma resulted most likely from the presence of a long alkyl chain of 35 carbon atoms in its chemical structure. At the highest dose tested (7.6 mg/kg/day over 28 days), annonacin decreased brain ATP levels by 44% and induced lesions in the basal ganglia and brainstem nuclei closely resembling those observed in patients with atypical parkinsonian syndromes [5]. Stereological cell counts showed significant loss of DA neurons in the substantia nigra (–31.7%) and cholinergic (–37.9%) and dopamine

and cyclic AMP-regulated phosphoprotein (DARPP-32)-immunoreactive GABAergic neurons (–39.3%) in the striatum, accompanied by a significant astroglial and microglial reaction. The pesticide rotenone, another mitochondrial complex I inhibitor, caused very similar brain lesions in the same paradigm of intoxication [14]. Unlike rotenone, however, no signs of systemic illness and no differences in bodyweight, food intake or general behavior were observed in annonacin-treated rats [5].

Interestingly, the estimated amounts of annonacin ingested by patients were significantly higher in Gd-PSP and Gd-PDC subgroups than in controls and PD patients and about 20-fold above the cumulative dose of 106 mg/kg that induced widespread neurodegeneration in the basal ganglia and mesencephalon of rats when infused intravenously over a period of 28 days [5]. However, in the absence of any knowledge of the bioavailability of annonacin after oral or intravenous administration, this comparison is only indicative of the threshold dose for neurotoxicity in humans [6].

Can Annonacin Intoxication Reproduce the Tau Pathology of the Disease?

Guadeloupean patients with atypical parkinsonism that came to autopsy had neuropathological features resembling those of PSP, in particular by an abnormal accumulation of tau, an axonal microtubule-associated protein [4]. Therefore, we investigated whether annonacin was able to induce changes in the intracellular distribution of tau. To this end, we used primary cultures of striatal neurons, because the striatum was one of the major sites of lesions in atypical parkinsonian patients [4] and in annonacin-treated rats [5]. Annonacin intoxication increased tau protein levels without increasing tau mRNA levels. It also caused the redistribution of the protein from the axons to the cell bodies. Retrograde transport of mitochondria from axonal processes to cell bodies and fragmentation of microtubules were also characteristic of neuronal demise induced by annonacin [12]. Taxol, a drug used for cancer therapy that works by stabilizing microtubules, prevented the somatic redistribution of both mitochondria and tau but failed to protect against neuronal death. Antioxidants that prevent the formation of reactive oxygen species produced during complex I inhibition by annonacin did not reduce the redistribution of tau and afforded no protection against cell death. Yet, both events were prevented by forced expression of the NDI1 nicotinamide adenine dinucleotide (NADH)-quinone-oxidoreductase, a yeast enzymatic system which can restore NADH oxidation and ATP production in mammalian cells in which complex I activity is impaired as the result of toxin exposure [25]. Consistent with this observation, stimulation of anaerobic glycolysis by glucose also prevented the redistribution of tau induced by annonacin [12].

In summary, experimental data demonstrate that very low concentrations of annonacin can induce pathological changes that are reminiscent of lesions observed in postmortem brains of patients with atypical parkinsonism in Guadeloupe. This suggests that annonacin may be critically involved in the pathomechanism of this disorder.

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Chapter 19
Cognitive Deficits in Parkinson's Disease

Eliana Roldan Gerschcovich and Kuei Y. Tseng

Introduction

Parkinson's disease (PD) is a progressive age-related neurodegenerative disorder characterized primarily by motor symptoms such as tremor, rigidity, and bradykinesia. These clinical manifestations do not emerge until the progressive damage of the dopamine (DA) system reaches critical level such as ~70% reduction of striatal DA terminals and ~50% decrease of DA neurons in the substantia nigra. It has been proposed that the delayed appearance of motor deficits associated with DA depletion is due to compensatory neuroadaptational mechanisms that normally takes place at presynaptic and postsynaptic levels after DA lesion [1].

On the other hand, PD patients also exhibit cognitive deficits, even in their earliest stages [1]. In addition to an increased risk for clinical dementia and depression [2], PD subjects also exhibit cognitive difficulties that resemble to those observed in patients with frontal lobe damage and mainly include the so-called frontal/executive deficits [3–12]. Impairments in executive functions such as working memory and attention deficits as well as difficulties in initiating goal-directed behaviors have been frequently observed in PD subjects [1]. Although L-DOPA therapy in early PD improves motor symptoms, its effects on cognitive performance are more complex and controversial [13]. Both positive and negative effects have been observed, suggesting that both motor and cognitive deficits in PD may or may not share a common neuropathophysiological substrate [1].

There is a general agreement of a cortico-basal ganglia-thalamocortical abnormality underlying the clinical manifestations in PD [14, 15]. In particular, motor deficits in PD have been traditionally associated with a dysregulation of the cortical control of subcortical circuits as a result of the progressive

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neurodegeneration of the nigrostriatal pathway and chronic DA depletion in the basal ganglia [14]. Whether a similar cortico-subcortical mechanism underlies the cognitive deficits in PD remains unclear. DA depletion in PD, however, is not restricted to subcortical brain regions. A reduction of DA and its metabolites has already been observed in several cortical areas in the PD brain [16, 17], suggesting that a decrease of cortical DA transmission could contribute to the onset of cognitive impairments observed in PD.

Epidemiology

The prevalence of dementia in PD is close to 30% bearing in mind that the incidence is 4–6 times greater compared to controls. The accumulative prevalence varies between 50 and 80% after 8–10 years of follow-up [18–20]. Rates of incidence for PD dementia (PDD) has been estimated between 95.3 and 112.5 in 1000 patients per year, that is, around 10% of PD patients may show clinical signs of dementia per year of disease progression [21, 22]. According to a recent study, it is estimated that after 5 years of disease onset, ~25% of PD patients will develop dementia. After 15 years ~50% of PD subjects will become PDD whereas ~35% will exhibit different degrees of mild cognitive deterioration [23]. It is estimated that only 15% of patients would show no evidence of cognitive deterioration after 15 years of PD onset [23]. Similarly, a follow-up study, occurring over the course of 8–10 years demonstrated that ~70% of PD patients exhibited significant cognitive impairments that include different degrees of dementia clinically evident in half of the population sampled [24, 25]. Thus, the mean duration from PD onset to the appearance of dementia is estimated to be ~10 years, with age as one of the major factors, having lower incidence of dementia in young patients [25–27].

The relative risks of developing dementia in patients with PD compared to subjects without PD vary from 1.7 to 5.9 times [22, 28–30]. The main associated risk variables include old age, the severity of motor symptoms such as rigidity, postural instability, and walking dysfunction, and the presence of mild cognitive deterioration at the beginning of the disease. Compared to the clinical profile of PD without dementia, cognitive deterioration in PDD typically has a slower progressive course [18]. Indeed, it has been documented that the mean annual rate of cognitive deterioration in the minimental test examination (MMSE) throughout 4 years of follow-up was 1 point in non-demented PD and 2.3 points in PDD [31]. A similar rate was reported in another longitudinal study with a mean reduction of MMSE of 4.5 points in 2 years [5]. Thus, cognitive deterioration in PDD resembles to that observed in patients with Alzheimer (AD) disease-related dementia.

Cognitive Features in Parkinson's Disease

A wide variety of cognitive impairments have been reported, even in early stages in the course of PD, including deficits in learning and memory, alterations in language and visuospatial skills as well as deficits in executive function [6, 8, 9, 18, 32–35]. In some cases, PD patients exhibit different degrees of amnesic profile clinically indistinguishable from that occurring in AD [33, 34]. For example, verbal fluency has been extensively studied in PDD [36–39]. This neuropsychological test assesses how well subjects activate frontal-related strategies to retrieve specific types of information. Relative to AD, patients with PDD exhibit greater impairments on the Initiation and Perseveration Scale of the dementia rating scale (DRS) [36] and deficits in concept formation as revealed by the "Conceptualization subscale" of the DRS, the Wisconsin Card Sorting Test (WCST), the "Similarities subtest" of the WAIS, and the Raven's Progressive Matrices [36–41]. Similarly, clinical evaluation of attention and vigilance states using the Serial 7 s of the MMSE and the DRS Attention subscale suggests that attention is also impaired in PDD [36–39, 42]. However, this latter is typically associated with a slower performance in reaction time and increased number of errors [40, 43]. Interestingly, attention deficits are not distinguishable in severity from those observed in patients with dementia with Lewy bodies (DLB) and AD [36–39]. On the other hand, memory deficits have been observed in 67% of PDD as compared to 100% in AD patients [32, 40]. PDD patients exhibit deficits at the level of recall performance in episodic memory, especially in the free recall condition [13, 42]. Significant deficits in memory performance in mild PDD have been reported using the Memory subscale of the DRS, yet the degree of impairments is less severe than that of patients with AD [36–39]. Interestingly, PD tends to show intact recognition memory and enhanced performance when given retrieval cues [32, 40]. Thus, recognition may be less affected than recall in mild-to-moderate PDD when compared to AD. In more severe dementia, however, the severity appears to match that seen in AD.

Drawing test performances, used to assess construction and praxis, are also impaired in PDD as evidenced by results obtained from a recent cohort in clinical trials [44]. A similar deficit was identified in a smaller study that failed to distinguish differences among patients with PDD, DLB, and AD, although there was evidence that the former two groups showed more 'planning' errors during the test [32, 37]. Other studies using design-copying tests all show impairments in PDD, yet no significant differences in drawing performance were observed between severe cases of PDD and AD patients [36–41, 45, 46].

Another cognitive feature, which has received little attention in PDD, is the assessment of visuospatial function without the demands of fine motor control. Using tests that evaluate the complex visuoperceptual and spatial functions revealed that PDD patients are more severely impaired than AD [8, 41]. Visual

discrimination, space-motion, and object-form perception were globally more impaired in PDD than in non-demented controls, but were not different from DLB. Compared to AD, PDD patients tend to perform worse in all perceptual scores [47]. Thus, PDD is associated with important visuoperceptual impairments similar to DLB, but different from AD.

Neuropsychiatric Symptoms in Parkinson's Disease

PD is also associated with several neuropsychiatric symptoms in addition to the motor deficits, which are relevant in determining the life quality in patients and their caregivers, the need for nursing home placement as well as the cognitive functioning and survival throughout the course of the disease [48, 49]. However, the underlying neurobiological mechanisms of such symptoms are not known. Using the neuropsychiatric inventory to describe the range of neuropsychiatric symptoms in a demographically representative sample of PD patients, Aarsland and co-workers [49] found that 61% of PD patients demonstrated psychiatric symptoms. In a community-based study applying formal diagnostic criteria, the rate of major depression in PDD was 13%, compared to 9% for patients without dementia, and 19% in DLB [50]. Both the severity and the prevalence of depression seem to be higher in PDD than in AD [41]. On the other hand, the prevalence of anxious mood in PDD and AD appears similar to that of mood depression. In fact, anxiety and mood depression occur at a similar frequency of 30–49% in PD, and both disturbances are frequently co-morbid, or occur in the same cluster of symptoms [50–56].

Hallucinations in PD patients are frequently observed in both population-based studies (25%) as in clinical samples (40%) assessed by the neuropsychiatric inventory [57, 58], with a substantially higher prevalence in PDD (45–65%) [57–60]. Hallucinations are in fact considered as a major predictor of subsequent dementia and nursing home placement. Hallucinations are more common in DLB, than in PDD, with a range of 60–80% [55, 59–61]. This high prevalence of hallucinations in PDD and DLB contrasts with relatively low rates reported in mild–moderate AD [50, 52, 60]. Overall, visual hallucinations are twice as frequent as auditory ones in PDD [47, 58, 59]. Finally, PDD patients sometimes exhibit delusions. Paranoid delusions, jealousy and 'phantom boarder' are the most frequent content [49–52, 55, 57, 59, 60] and typically cause distress and reduce life quality in PDD [62]. Both delusion and hallucination symptoms often coexist in PD.

Difficulties in the diagnosis of depression arise not only in differentiating depression from PD but also in differentiating it from apathy and fatigue syndromes [49]. The underlying pathophysiology of apathy and fatigue remains poorly understood. In contrast to what is typically observed in depression, apathy in non-depressed patients with PD is characterized by lack of motivation and initiative as well as anhedonia, which is sometimes combined with

hopelessness and low mood [49]. The frequency of apathy in PDD is ~25% [50], yet a recent study analyzing a large sample of mild–moderate PDD patients reported a higher incidence (up to 54%) as compared to ~15% in patients without dementia [49, 51, 57].

Overall, neuropsychiatric symptoms are common in PDD. Hallucinations are one of the few features that can be used to distinguish between DLB/PDD and AD. Although the manifestation of psychiatric symptoms could be associated as risk factors for developing dementia, they do not appear to be useful in differentiating PDD from DLB and AD in individual cases.

Functional Changes Underlying Cognitive Deficits in Parkinson's Disease

Neuroimaging studies indicate that the degree of structural alterations in PDD include temporal lobe atrophy and bilateral reductions in the occipital lobes [32, 63–65]. Interestingly, the pattern of regional atrophy in PDD, PD, and DLB resembles to that observed in AD [32, 63–70]. On the other hand, proton MR spectroscopy studies using lactate/*N*-acetylaspartate ratio analyses indicate that the oxidative metabolism may be impaired in PD, in particular, in PDD patients [71]. Summerfield and co-workers [69] found a significant decrease in the ratio of *N*-acetylaspartate in the occipital lobes of PDD when compared to PD without dementia. Furthermore, SPECT and PET studies provide evidences of functional disruption in the PDD brain as revealed by reduction of cerebral blood flow (CBF) in different brain areas including the temporal, parietal, posterior cingulate, and occipital cortices [72–80]. In contrast to what is observed in PDD [76, 78], PD patients without dementia exhibit a more pronounced decrease in CBF that seems to be limited to the frontal lobe [72, 74]. Thus, the pattern of cortical hypofunction in the PDD brain resembles to that of AD, although the changes found in AD are typically more pronounced [80].

Perhaps there is a link between common functional losses in cognitive functions associated with AD and PD as they might be related to a dysregulation of the mesocorticolimbic DA system. For example, a study by Volkow and colleagues suggests that the levels of striatal DA D2 receptors are correlated with the degree of frontal cortical-dependent cognitive performance [81, 82]. A similar relationship was found in PD [83]. In fact, activation of the mesolimbic/mesocortical DA system is context dependent and related to attention and salient stimuli [84–86]. Furthermore, DA regulation of the frontostriatal system has been associated with a wide range of cognitive tasks such as working memory, planning, and the organization of goal-directed behaviors [84–86]. Indeed, DA depletion or blockade within the frontostriatal pathway

impairs working memory performance in rats and non-human primates [87–89]. Similarly, deficits in working memory-dependent tasks in PD patients are correlated with impairments in frontal cortical/corticostriatal activation [9, 90–92]. Thus, therapeutic interventions aiming to restore the mesocorticolimbic DA function could improve cognitive performance in PD.

Genetics of PDD

Parkinsonism and the associated dementia are occasionally inherited. The effect of genetic predisposition as a risk factor has only been studied systematically for APO-E genotypes, and the results have been conflicting. A positive correlation of E2 or E4 alleles with PDD was reported in some studies [29, 93], whereas a negative association with E4 was found in a number of others [94–97]. Dementia has been reported in familial forms of PD such as PARK1 and PARK8 [98–101]. Dementia is rare in PARK2, PARK6, and PARK7. There is some evidence for familial aggregation of dementia in PD [102]. Increased frequency of dementia in the relatives of PD patients with dementia has been found in some [103], but not in all cases [104–106].

Genetic abnormalities involving the α-synuclein gene on chromosome 4 may lead to either PD or PDD [100, 101, 107–109]. Mendelian genetic studies suggest that even with autosomal-dominant mutations, clinical phenotypes often differ [100, 107, 109]. For instance, extra copies of the α-synuclein gene may lead to either PDD or DLB. Because the basic pathophysiological mechanism underlying genetic and sporadic diseases is often similar, it is plausible that sporadic PDD and DLB share the same pathogenesis for the formation of Lewy bodies, in particular, when genetic abnormalities of the α-synuclein gene are strongly associated [107].

PD with Lewy bodies as an etiologically distinct movement disorder was strongly reinforced by the identification of α-synuclein mutations in autosomal-dominant PD and by the demonstration that pathologically altered forms of α-synuclein are the major building blocks of the filaments aggregating to form Lewy body and Lewy neurite pathology [20, 110, 111]. This notion was supported by the subsequent identification of additional α-synuclein mutations and Lewy body pathology in a variety of clinical manifestations of PD (without dementia), PDD, and DLB [20, 110, 111]. Each of these clinical entities is characterized by widespread Lewy bodies and related α-synuclein pathologies such as dystrophic Lewy neurites [100, 101]. Thus, it is now clear that the accumulation of pathological species of α-synuclein and the deposition of α-synuclein fibrils into inclusions result in the formation of diagnostic signatures of α-synucleinopathies. More importantly, there is evidence indicating that these pathological changes maybe critical in determining the onset and progression of PD and related disorders.

Dominantly inherited forms of AD (mutations in the APP and presenilin-1 genes) often have Lewy body pathology, suggesting that genetic abnormalities unrelated to α-synuclein may also promote aggregation of α-synuclein [112]. In fact, brains from Down syndrome subjects often contain Lewy bodies [113] and mutations in leucine-rich repeat kinase 2 (LRRK2) gene, a common genetic cause of PD (sometimes with dementia) that may lead to α-synuclein, tau, or ubiquitin pathology (with or without Lewy bodies) [114]. Perhaps susceptible genes may cause abnormal protein processing or other types of cellular damage leading to aggregation of various CNS proteins, including those identified for DLB and PDD [115]. In fact, there are multiple genetic factors that can trigger the formation of Lewy bodies, regardless of the disease phenotype. Thus, the combination of dementia and PD does not always reflect on α-synuclein pathology. Mutations of the tau gene on chromosome 17 may also show PDD or DLB clinical phenotypes [116].

Pathological Correlations

The presence of widespread Lewy bodies differentiates the Lewy body disorders from other dementia subtypes. Cortical Lewy bodies and Lewy neurites are often widespread in PDD and DLB, and are typically correlated with the severity of dementia [106, 117, 118]. However, there are no hallmark neuropathological features that distinguish PDD from DLB as most patients die with end-stage disease, typically when several brain regions are diffusely compromised [106, 117, 118]. The pathophysiological substrates underlying PDD and DLB are heterogeneous, including neuronal loss and degeneration within the basal forebrain-cholinergic system, making the two clinical phenotypes indistinguishable [119]. Therefore, the presence of β-amyloid pathology and its association with the diverse clinical phenotype remain unclear [120, 121].

α-Synuclein aggregates into fibrils in Lewy bodies and Lewy neurites in PDD, DLB, and PD. The structure of the Lewy body is indistinguishable in all these three conditions, with α-synuclein as its principal pathological feature [116, 122]. Solubility and epitope studies show similar features in α-synuclein among diseases. Although the brainstem and olfactory system are the first and most common regions to undergo neurodegeneration in PD, the disorder is often clinically unapparent at that stage, and only becomes clearly symptomatic when the substantia nigra and other midbrain nuclei are affected. Later, multiple additional brain regions become compromised as the disease progresses so that patient's clinical manifestations commonly extend beyond those attributable to the nigrostriatal system alone [110]. As such, PD, DLB, and PDD may be different points of a continuum with motor and non-motor features reflecting the regional burden and distribution of pathology. Factors that determine the underlying pathophysiology for the emergence of symptoms in DLB, PDD, and PD are not well understood. Age may play a role in

determining the appearance of cognitive symptoms in PD, as younger individuals are more likely to exhibit motor deficits with subtle or a lack of cognitive impairments [123].

Hallucinations in DLB are associated with Lewy body counts in posterior temporal regions [124]. The greater executive dysfunction in DLB has been associated with disruption of medial temporal lobe projections to frontal cortices. Similarly, differential striatal pathology may account for some of the differences in motor features, in motor responses to medications with differences in the burden of Lewy pathology, the severity of DA loss, and DA receptor upregulation [125]. Preliminary results from a recent autopsy study (*Aarsland et al. 2006, Mov Dis, 21: S96*) indicate that PDD shares a similar pattern of brain pathology to that of DLB, in particular if dementia began within 10 years of PD onset. If the onset of dementia occurred >10 years after the emergence of PD symptoms the morphological changes were less pronounced.

As discussed above, it is clear that there are multiple reasons to implicate Lewy bodies and pathological species of α-synuclein in PD, PDD, and DLB. However, the mechanism by which α-synuclein leads to neuronal death remains unknown [126, 127]. α-Synuclein is abundant in the normal brain at the synaptic terminal [127]. It may regulate DA release or interact with other molecules to protect presynaptic nerve terminals from injury [128, 129]. Because Lewy body pathology is composed of fibrillar α-synuclein, and several autosomal-dominant mutations in α-synuclein lead to enhanced rates of protein fibrillization, this conformational change in the structure of α-synuclein may render its neurotoxicity. Some investigators have proposed that small, prefibrillar oligomers of α-synuclein are the toxic species leading to neuron dysfunction and degeneration. Conversely, the sequestration of this synaptic protein into inclusions may result in the loss of a critical biological function leading to cell toxicity. α-Synuclein accumulations in synaptic terminals may lead to lysosomal leakage and signaling abnormalities. Serine 129 (Ser129) is an important phosphorylation site for α-synuclein in all the Lewy body disorders [130]. Antibodies specific to phosphorylated α-synuclein have been shown to specifically recognize Lewy bodies and Lewy neurites. DA is another factor that affects α-synuclein structure, inducing α-synuclein to form soluble oligomers and reducing insoluble fibrils [131, 132]. The presence of truncated C-terminal forms of the α-synuclein is likely to facilitate the initiation of α-synuclein aggregation, which in turn may promote disease progression [133]. Furthermore, proteins not directly related to α-synuclein, such as heat shock proteins and other chaperone proteins, are undoubtedly affected and linked to the pathogenesis of LBD [134]. Inflammation is also likely to be a relevant contributor to the neurodegenerative cascade in LBD [128, 129]. For example, the common co-occurrence of α-amyloid and α-synuclein raises the possibility of a major pathogenic mechanism between APP and α-synuclein aggregation [135]. Thus, the presence of Lewy body-type degeneration in cortical regions is the main pathological correlate of dementia in PD [106].

Summary and Conclusions

It becomes clear that dementia in PD best correlates with Lewis body pathology, and that PDD can be designated as a Lewis body-associated dementia. Although AD-type pathology frequently co-exists with PDD, the clinical features of PDD include insidious onset and a slowly progressive course of cognitive impairments in attention, executive, and visuospatial functions as well as memory. Hallucinations, delusions, apathy, and mood changes are frequently associated behavioral features in PDD.

Overall, the profile of cognitive and behavioral symptoms in PDD and DLB is very similar. The defining feature of PDD is that dementia develops in the context of established PD. Therefore, the diagnosis of idiopathic PD before the development of dementia symptoms is essential. Diagnosis of dementia must be based on the presence of cognitive deficits that affect patients normal functioning in at least two of the four core domains: attention, memory, executive, and visuospatial functions. Although patients with AD exhibit major memory impairments as compared to a more prominent executive dysfunction in PDD, there are some individual differences that may vary from patient to patient. Neuropsychiatric and behavioral symptoms are frequent, but are not invariable.

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Part V
Pharmacological and Non-Pharmacological Treatments in Parkinson's Disease

Chapter 20
Dopamine Replacement Therapy in Parkinson's Disease: Past, Present and Future

M.A. Cenci and P. Odin

L-DOPA Pharmacotherapy in Perspective

Until the 1960s, the only available pharmacotherapy for Parkinson's disease (PD) consisted in anticholinergic drugs, whose positive symptomatic effects were discovered serendipitously [1]. This category of drugs is particularly effective in alleviating tremor, but also causes significant side effects due to the blockade of muscarinic receptors in the autonomic and central nervous system. The modern era of PD pharmacotherapy started about 50 years ago and was made possible by two scientific breakthroughs. Arvid Carlsson and collaborators discovered that dopamine (DA) was a centrally active neurotransmitter and that DA depletion by reserpine produced a syndrome very similar to parkinsonism in animals [2]. A few years later, Hornykiewicz and collaborators discovered DA deficiency in the brains of PD patients [3] and paved the way for the use of DA precursor, L-3,4-dihydroxyphenylalanine (L-DOPA) as a replacement therapy [4]. L-DOPA had an impressive efficacy on all the motor features of PD (resting tremor, rigidity, akinesia and postural instability) [5] and seemed to provide the ultimate treatment for this condition. It soon became apparent, however, that this treatment was not devoid of complications. Already at the beginning of the 1970s, abnormal involuntary movements (dyskinesia) were reported as a common, dose-limiting side effect of L-DOPA pharmacotherapy [6–9]. Involuntary movements could occur very early after the initiation of L-DOPA treatment, but their incidence and severity increased with time. Along with the clinical use of L-DOPA grew an awareness of its limitations.

It is now well established that the response to L-DOPA changes during the progression of PD [10]. As the disease becomes more severe, the need for symptomatic medications becomes larger. Thus, both the total dosage and the

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number of L-DOPA doses per day are usually increased a few years after treatment initiation [11]. At this point, patients start to exhibit abnormal involuntary movements (dyskinesia) and motor fluctuations. These complications affect approximately 40% of the patients after 4–6 years of L-DOPA therapy [12] and up to 90% of the patients by 10 years of treatment [13, 14]. The most common pattern of L-DOPA-induced dyskinesia consists of choreiform movements that are most severe at the time when the drug is producing the maximal relief of parkinsonian motor symptoms, hence the term "peak-of-dose" or "on" dyskinesia [15]. In some patients, involuntary movements are most prominent at the beginning and at the end of the L-DOPA dosing cycle, a pattern referred to as "diphasic dyskinesia". This form of dyskinesia typically manifests as stereotypic or ballistic movements mixed with dystonia and is particularly severe in the legs [16]. Motor fluctuations appear as rapid transitions from good motor function ("on" phase) to severe parkinsonian immobility ("off" phase) [17, 18]. The earliest and most common type of motor fluctuation consists in a decreased duration of the effect of single L-DOPA doses, termed "wearing-off phenomenon" or "end-of-dose deterioration". This usually calls for an adjustment in the treatment regimen, where the daily L-DOPA dosage becomes distributed into a larger number of administrations per day. With increased dosage fractionation, the response to L-DOPA becomes more erratic [19], and fluctuations between "on" and "off" time can be unpredictable [11]. Pharmacokinetic problems are certainly insufficient to explain the occurrence of dyskinesia and motor fluctuations [20]. In animal models of PD, treatment with L-DOPA induces an exuberant activation of nuclear signalling pathways in striatal neurons and altered patterns of neuronal discharges in the deep basal ganglia nuclei. These alterations are causally linked with the occurrence of abnormal involuntary movements, and similar phenomena are likely to occur in human patients (reviewed in Cenci [21] and Cenci and Lindgren [22]). Maladaptive neuroplasticity is assumed to play a major role in the induction of motor complications by L-DOPA [23, 24]. This assumption is supported by the prominent impact of young age at PD onset as a risk factor for dyskinesia [13]. Indeed, the potential for plastic changes is more pronounced in a younger brain [24].

Dyskinesia and motor fluctuations are not the only problem associated with the use of L-DOPA. The stimulation of DA receptors in mesocorticolimbic regions can cause psychotic-like symptoms (hallucinations, vivid dreams, confusion) [10]. A matter of even greater concern is the limited efficacy of L-DOPA against a range of severe motor, autonomic and cognitive deficits that plague patients in an advanced disease stage [25]. Moreover, L-DOPA is unable to halt the progression of neurodegeneration, and neuroprotection can be regarded as the prime unmet need in the treatment of PD [10, 26]. Despite all these limitations, L-DOPA is still recognized as the most efficacious drug to alleviate the signs and symptoms of PD [27] and it is also the cheapest treatment [10]. At present, there is no realistic prospect that DA replacement therapy with L-DOPA will be radically substituted for by newer strategies, such as

neuroprotective treatments, intracerebral transplantation or deep-brain stimulation. Although standard dosing regimens are currently being debated, very few specialists would question the utility of L-DOPA per se. Clinical pharmacological research on PD is now focusing on two issues: (i) How can L-DOPA delivery methods be optimized to reduce the occurrence of dyskinesia and response fluctuations? (ii) Can L-DOPA be combined with non-dopaminergic treatments to improve its efficacy and alleviate non-DA-dependent symptoms? While the first issue is likely to be resolved in a few years' time, the problems posed by DOPA-resistant symptoms in PD remain challenging. The pathophysiology of cognitive, psychiatric and autonomic features in PD is complex and poorly understood, and valid animal models are yet lacking. A short summary of this difficult area will be given at the end of this chapter, which will otherwise mainly focus on dopaminergic treatments to manage the motor aspects of PD.

Current Options for a Dopaminergic Pharmacotherapy in PD

Standard L-DOPA pharmacotherapy consists of tablets for oral administration, which are usually taken from three to eight times per day depending on the individual response and disease stage. L-DOPA is rapidly absorbed from the small intestine, but its absorption depends on the rate of gastric emptying and on the pH and amino acid concentration of the gastric contents [28]. Plasma concentrations usually peak between 1 and 2 h after an oral dose, and the plasma half-life is usually between 1 and 3 h [29, 30]. Unfortunately, only about 1–3% of administered L-DOPA actually enters the brain unaltered, the remainder being metabolized extracerebrally, predominantly by decarboxylation to DA, which does not penetrate the blood–brain barrier (BBB). To reduce the extracerebral conversion of L-DOPA, standard L-DOPA preparations also contain an inhibitor of DOPA decarboxylases which does not enter the BBB, such as carbidopa or benserazide [31]. Typically, L-DOPA achieves a satisfactory control of PD motor symptoms during the first 3–5 years of treatment. As the disease advances, the benefit produced by single L-DOPA doses becomes more short-lived, and the window between the threshold dose reversing parkinsonism and that inducing dyskinesia becomes narrower [32]. In this stage, the majority of the patients also exhibit dyskinesia during the "on" periods (Fig. 20.1). The resulting disability can leave patients unable to perform normal daily activities such as sitting, eating or walking [33]. In this complicated stage, three types of interventions currently represent the most effective options, i.e. deep-brain stimulation (see Gubellini et al., this book), continuous duodenal L-DOPA administration (extensively discussed in the next section of this chapter) or subcutaneous apomorphine infusion.

Apomorphine is a broad-spectrum DA receptor agonist, whose efficacy against Parkinson symptoms was first demonstrated in 1951 [34]. The compound has conspicuous gastrointestinal side effects (nausea, vomiting) due to

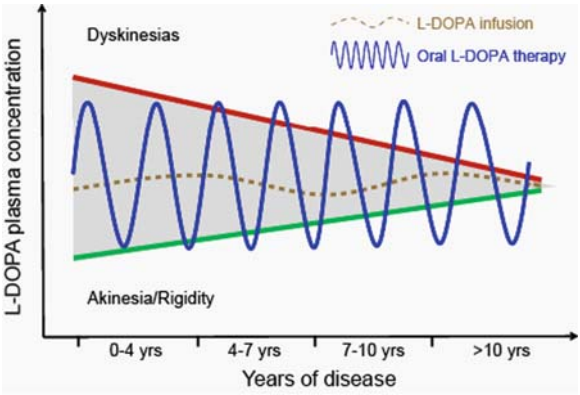

Fig. 20.1 Theoretical model illustrating how the therapeutic window of L-DOPA changes during the progression of PD and how this impacts on the motor response to L-DOPA. The *upper oblique line* indicates the threshold L-DOPA concentration above which patients exhibit dyskinesia; the *lower line* indicates the threshold concentration required to reverse PD motor features. The *shaded area* indicates the range of L-DOPA concentrations at which the patient exhibits an "on" response without dyskinesia. In early disease stages, standard regimens of L-DOPA pharmacotherapy achieve a good and stable control of the clinical status. As the disease advances, the dose of L-DOPA required to provide symptomatic benefit becomes larger, while the threshold L-DOPA concentration inducing dyskinesia becomes smaller. As the therapeutic window of L-DOPA narrows, the daily medication-induced swings in plasma L-DOPA levels (*blue line*) cause pronounced fluctuations between "on" time with dyskinesia and "off" time with severe parkinsonism. At this stage, only a continuous infusion of L-DOPA (*brown dashed line*) has a chance to maintain an acceptable motor status. The progression from a stable response to a complicated motor response to L-DOPA shows pronounced individual variations and occurs much faster in patients with young-onset PD

the stimulation of peripheral DA receptors. A broader clinical application of apomorphine became possible only after discovering that domperidone (a DA receptor antagonist that does not cross the blood–brain barrier) could be coadministered with apomorphine to block its peripheral adverse reactions [35]. Since the late 1980s apomorphine is in general use for PD treatment, most often in the form of subcutaneous (s.c.) infusions or injections [36, 37]. Apomorphine is, together with L-DOPA, the most efficacious treatment for PD motor symptoms. The effects of these drugs seem to be comparable, but their pharmacokinetics are considerably different. After a s.c. injection apomorphine has a half-life in distribution phase of about 5 min, leading to a clinical effect after 5–10 min. The biological half-life in elimination phase is around 33 min and the effect duration about 60 min [38]. Because of its short duration of action, apomorphine is currently administered by continuous subcutaneous infusion via a specially designed portable infusion pump. This treatment is best suited for the severely disabled patient who has a good L-DOPA response, but whose condition is dominated by prolonged or frequent "off" periods despite optimized oral drug treatment [39–41]. Apomorphine infusion is also

indicated for patients with early-onset PD affected by disabling peak-dose dyskinesias or disabling "off" phases occupying a significant part of the day [40]. In a review of clinical outcomes of continuous apomorphine infusion therapy [42], including 11 published studies (mainly [38, 41, 43–48]), the treatment resulted in an average 61% reduction of the time spent in the "off" phase after a mean follow-up period of 21 months. The daily L-DOPA dosage could be reduced by about 39%, and dyskinesia was significantly improved [45, 49]. The effects of apomorphine infusions seem to be stable over long-term follow-up [50]. The most frequent problem associated with apomorphine infusion is the formation of subcutaneous nodules. This occurs in almost all treated patients and may cause the therapy to be discontinued. This problem can be prevented or delayed by avoiding apomorphine concentrations higher than 5 mg/ml and by changing the infusion area at least twice per day. Dopaminergic psychotic-like side effects are also a matter of concern. The prevalence of psychotic complications is, however, not higher with apomorphine compared to other dopaminergic therapies. Haemolytic anaemia has been reported in about 3% of the treated patients [44, 47]. Beside pump treatment, apomorphine can also be given as bolus s.c. injections in order to terminate "off" periods occurring in spite of an optimized peroral therapy. The effective dose is the lowest apomorphine dose producing a full antiparkinson effect (typically around 2–4 mg), and this has to be titrated in each patient. The injections are delivered to the patient's lower abdomen or outer thigh upon the first signs of an "off" episode. Domperidone is given during the first days of treatment and can later be tapered off in most patients. The efficacy of this treatment has been demonstrated in a number of studies [51].

In addition to apomorphine, a relatively large number of compounds acting as direct DA receptor agonists have been introduced in the treatment of PD during the past 35 years. The prototype of these agonists is bromocriptine, an ergot derivative with agonist activity at the D2 receptor, but additional DA agonists (both ergot and non-ergot derivatives) are available (Table 20.1). These DA agonists differ between each other in terms of pharmacokinetics and side effect profile, but they have similar DA receptor specificity, acting on the D2/D3 receptor class. Moreover, they all have a much longer duration of action than L-DOPA. Initial treatment of PD with these agonists has been consistently reported to reduce the incidence of dyskinesia and motor fluctuations [52]. Dopamine agonists have therefore become first-line agents for de novo treatment of young PD patients in many countries (see e.g. German National Guidelines for treatment of Parkinson's disease, www.dgn.org). Another indication is represented by patients with motor fluctuations, where the addition of a DA agonist to L-DOPA reduces the time spent in the "off" condition [53, 54]. This category of compounds is, however, not devoid of untoward effects. The incidence of psychiatric side effects, such as hallucinations, delusions, confusion or impulse control disorders, is overall larger for these compounds compared to L-DOPA [55]. Ergot-derived DA agonists can induce pulmonary or retroperitoneal fibrosis [56]. Moreover, an association has

Table 20.1 DA agonists currently used in the treatment of PD

Substance	Type	T1/2 (h)	Elimination
(a)			
Apomorphine	Non-ergot	0.5	
Bromocriptine	Ergot	6	Hepatic
Cabergoline	Ergot	65	Hepatic
α-Dihydroergocryptine	Ergot	15	Hepatic
Lisuride	Ergot	2–3	Hepatic/renal
Pergolide	Ergot	7–16	Hepatic/renal
Pramipexole	Non-ergot	8–12	Renal
Ropinirole	Non-ergot	6	Renal
Rotigotine (patch)	Non-ergot	5–7	Renal
Equivalence doses	Single dose		
(b)			
L-DOPA	100 mg		
Apomorphin	3–5 mg		
Bromocriptine	10–15 mg		
Cabergoline	1.5–2 mg		
α-Dihydroergocryptine	20–40 mg		
Lisuride	1 mg		
Pergolide	1 mg		
Pramipexole	0.7–1 mg		
Ropinirole	3–5 mg		
Rotigotine (patch)	4 mg/24 h		

been recently demonstrated between treatment with pergolide or cabergoline and an increased risk of newly diagnosed cardiac valve regurgitation [57, 58]. The effect was attributed to the agonist activity exerted by pergolide and cabergoline at 5-hydroxytryptamine 2B (5-HT 2B) receptors. Indeed, stimulation of 5-HT 2B receptors has mitogenic properties on cardiac fibromyoblasts, potentially leading to valvular fibroplasia [59, 60].

In addition to DA receptor agonists, other dopaminergic treatments for PD include inhibitors of enzymes that inactivate DA, i.e. monoamine oxidase B (MAO-B) [61] and catechol-*O*-methyl-transferase (COMT) [62]. These enzyme inhibitors are administered together with L-DOPA in order to prolong its effects and reduce the time spent in "off". Moreover, MAO-B inhibitors also have mild efficacy as a monotherapy and can delay by several months the need for L-DOPA [63]. Interestingly, the MAO-B inhibitor rasagiline can activate neurotrophic factor signalling [64], raising hopes for a disease-modifying action in human PD [65].

Despite the rich drug arsenal reviewed above, the vast majority of PD patients will require DA replacement with L-DOPA at some point during the course of the disease. Multiple mechanisms can be envisaged to explain the superior therapeutic efficacy of L-DOPA compared to any other dopaminergic drug: (i) DA formed from exogenous L-DOPA can be stored in residual nigrostriatal DA neurons, which will release it when and where needed;

(ii) following its conversion to DA, the drug stimulates all types of DA receptors; (iii) L-DOPA is also a precursor of noradrenaline, and a synergism between noradrenergic and dopaminergic effects may add to the therapeutic benefit; (iv) L-DOPA may influence motor behaviour also through its metabolites, which can activate trace amine receptors [27]; (v) finally, some authors have suggested that L-DOPA can act as an endogenous neuromodulator [66], although the therapeutic relevance of this potential mechanism is unclear.

The Concept of Continuous Dopamine Stimulation and the Ways to Achieve It

Many of the problems caused by L-DOPA pharmacotherapy are believed to stem from its mode of administration rather than from the drug itself. In PD patients, standard treatment regimens cause fluctuations in the extracellular concentrations of L-DOPA and DA within the brain. Intermittent surges and troughs in brain DA tone are at variance with the physiology of DA neurotransmission. Nigrostriatal DA neurons have a tonic "pacemaker-like" firing activity [67] with superimposed bursts of action potentials that are triggered by incoming stimuli (in particular, reward or prediction of reward [68]). Phasic activity bursts induce, however, very modest increases in extracellular DA levels because of the high efficiency of presynaptic control mechanisms, consisting of inhibitory DA autoreceptors and DA reuptake at nigrostriatal axon terminals [69]. Accordingly, extracellular DA concentrations are maintained within a narrow physiological range in a healthy striatum [69]. It has therefore been proposed that a continuous supply of L-DOPA and/or a continuous stimulation of DA receptors (collectively referred to as "continuous DA stimulation", CDS) would be required to adequately reproduce the physiological features of nigrostriatal DA transmission [70–72]. A first experimental verification of this concept was provided by Thomas Chase and collaborators. Using rats with 6-OHDA lesions, these investigators compared the effects of the same daily L-DOPA dose when administered through bidaily i.p. injections or continuous i.p. infusion. Only the pulsatile treatment resulted in upregulation of molecular markers that were later found to correlate with the development of dyskinesia, such as increased levels of dynorphin and glutamic acid decarboxylase (GAD) in the basal ganglia [73, 74]. Moreover, the two modes of L-DOPA administration resulted in different patterns of 2-deoxyglucose uptake in the basal ganglia [75]. On a behavioural level, only the pulsatile administration caused marked sensitization of rotational responses to dopaminergic agents [74, 76]. In addition to these experimental data, in vivo imaging studies in PD patients strongly support the association between rapid and large changes in striatal DA release and the occurrence of dyskinesia [77, 78]. Moreover, it has been proposed that the lower dyskinesiogenic potential of the DA agonists compared to L-DOPA depends on their longer duration of action [72, 79].

Because of all these considerations, intense efforts have been focused on developing strategies for CDS in the last 20 years. The different approaches used are reviewed below.

Continuous Duodenal or jeujenal Infusion of L-DOPA

The first studies describing intravenous delivery of L-DOPA were published in 1975 [80] and were soon followed by several other reports showing an improvement of motor fluctuations [81]. In most cases the patients were, however, only treated for a few days. It proved practically difficult to give L-DOPA intravenously over longer times. The first experiences with intraduodenal L-DOPA infusion were published in 1986 [82], reporting an effect comparable to i.v. L-DOPA delivery. This was later confirmed in several other studies (reviewed in [83]). Duodenal delivery methods for broad clinical application have been developed mainly in two different centres, the University of New Brunswick (NJ, USA) and the University of Uppsala (Sweden). A collaboration between the Department of Neurology and the Department of Galenic Pharmacy at the University of Uppsala led to the development of Duodopa® [83]. Duodopa is a combination of L-DOPA (20 mg/ml) and carbidopa (5 mg/ml) in the form of a pseudoplastic gel. Duodopa is delivered with portable infusion pumps. For short-term therapy a nasoduodenal catheter is used. For long-term treatment a catheter is inserted into the duodenum by surgical intervention (percutaneous endoscopic gastrostomy, PEG, or jejunostomy, the latter being performed only in a few cases). Controlled studies have shown that Duodopa achieves a stabilization of both plasma L-DOPA concentrations and clinical status (Fig. 20.1), producing a strong reduction of motor fluctuations and an overall increase of time spent in "on" [83]. A blinded randomized cross-over study comparing Duodopa monotherapy with individually optimized peroral therapy has demonstrated an increase in daily "on" time from 81 to 100% and an improvement in health-related quality of life upon treatment with Duodopa [84]. In a German study, 13 patients treated with Duodopa experienced a mean 82% reduction of time spent in "off" per day, whereas the time spent in "on" without dyskinesia increased from 30 to 90%, and peak-of-dose dyskinesia virtually disappeared during a mean follow-up time of 6 months [85]. Similar effects have been reported in other independent studies [86, 87]. Duodopa is initially given only during daytime. In patients experiencing night-time problems with Parkinson symptoms and suboptimal sleep, a 24-h treatment can bring significant improvements without inducing further side effects or tolerance [88]. Adverse events of Duodopa therapy are related to the infusion method. These include dislocation or occlusion of the duodenal catheter or leakage in the infusion system. The main drawback associated with L-DOPA infusion therapy does not, however, lie in these complications but in its high costs, which would become prohibitive for the health care systems in most

countries if this treatment were to be routinely indicated to patients with motor fluctuations.

Transdermal Drug Delivery

Transdermal drug delivery is a relatively recent development tried for several DA agonists. A transdermal patch formulation of the non-ergolinic DA receptor agonist, rotigotine, is indicated either as a monotherapy in the treatment of early-stage PD or as an adjunct to L-DOPA across all disease stages. Transdermal rotigotine has been shown to be superior to placebo in patients with early-stage and advanced PD, although noninferiority to the oral DA receptor agonists, ropinirole or pramipexole, was not consistently demonstrated [89]. The patch delivery option is advantageous in several situations, such as (i) when peroral delivery is contraindicated by specific medical conditions (dysphagia, gastrointestinal side effects of peroral drugs, perioperative conditions and gastrointestinal resorption); (ii) in patients with compliance problems; (iii) in patients with Parkinson-related sleep disturbances in late night/early morning. The most common side effects of rotigotine are the skin reactions, which occur very frequently and lead to termination of the therapy in about 5% of the treated patients [89, 90]. A transdermal approach is now being developed also for delivering L-DOPA in the form of a soluble ester (see www.neuroderm.co.il/overview.html). Also lisuride and apomorphine are being investigated regarding the possibility of a transdermal delivery [91, 92].

"Enzyme Replacement Therapy" by Gene Transfer

Dopamine is synthetized in two enzymatic steps from dietary tyrosine. Tyrosine is first converted to DOPA by tyrosine hydroxylase (TH), and DOPA is then converted to DA by aromatic amino acid decarboxylase (AADC). The activity of the TH enzyme is dependent on the presence of the reduced form of the co-factor tetrahydrobiopterin, synthetized in three steps from guanosine triphosphate (GTP), where the first and rate-limiting step is catalysed by GTP cyclohydrolase 1 (GCH1) (reviewed in [93]). The vast majority of TH and GCH1 proteins in the striatum are located in DA fibres. Although AADC is mainly found in DA fibres, this enzyme is also expressed in serotonergic neurons, glial and endothelial cells and in some striatal neurons (reviewed in [94]). Accordingly, in 6-OHDA-lesioned rats, endogenous DA synthesis is totally lost in the striatum after a severe DA-denervating lesion, whereas the capacity for decarboxylation of exogenous L-DOPA is largely preserved (reviewed in [93]).

One proposed strategy for CDS in PD consists in introducing the genes coding for DA-synthetizing enzymes directly into the striatum by either of two principles: (i) implants of genetically modified cells (ex vivo gene transfer)

or (ii) viral vector-mediated gene delivery to resident cells (in vivo gene transfer). No satisfactory outcome has yet emerged from ex vivo gene transfer approaches, whereas in vivo gene transfer of DA-synthetizing enzymes has achieved very encouraging results in animal models of PD. Multiple striatal injections of recombinant adeno-associated virus (rAAV) coding for TH and GCH1 result in an efficient DOPA synthesis in the striatum associated with significant behavioural improvement in 6-OHDA-lesioned rats [95]. Similar approaches have been recently tried with success also in non-human primate models of PD [96]. In experiments where gene transfer of TH and GCH1 was applied to dyskinetic rats, the severity of L-DOPA-induced dyskinesia (as evoked by challenge i.p. injections of the drug) gradually and dramatically decreased during the post-surgical phase. This was paralleled by reversal of maladaptive molecular changes that had been induced and maintained by pulsatile peripheral administration of L-DOPA, such as the upregulation of ΔFosB and opioid precursor genes in striatal neurons [97]. These data are noteworthy because they provide evidence that CDS can not only prevent but also erase L-DOPA-induced plastic neural changes causing aberrant motor responses. In the field of in vivo gene transfer, the approach closest to clinical application is the transduction of striatal neurons with a gene coding for AADC. This approach is meant to locally enhance the capacity for L-DOPA conversion in the motor part of the striatum, thus reducing the dose requirement for peripheral L-DOPA. This may provide a way to reduce non-motor side effects of the pharmacotherapy, in particular, the psychiatric complications related to high DA levels in mesolimbic regions. An efficient method for striatal AADC gene transfer using adeno-associated viral vectors (AAV) was first characterized in non-human primates' models of PD [98] and then evaluated in patients. A phase I safety trial has now been completed, showing that this approach is well tolerated and results in sustained putaminal transgene expression [99]. Differently from the TH/GCH1-based methods, AADC gene transfer does not lift the requirement for peripheral L-DOPA administration, and it would seem unlikely to reduce the incidence of motor complications when compared with standard L-DOPA pharmacotherapy. Nevertheless, the recently completed clinical trial with AAV–AADC [99] provides a proof of principle for the feasibility of in vivo gene transfer in PD.

Neural Transplantation

Intrastriatal grafting of embryonic DA neurons from the ventral mesencephalic (VM) primordia has been extensively characterized in animal studies and also tested with some success in human PD patients. Intrastriatal VM grafts can be viewed as a cell-based approach to CDS because the transplanted DA neurons are able to provide a source of continuous DA release. In vivo monitoring of extracellular DA concentrations in the tissue surrounding VM transplants in

the rat has demonstrated normal, nonfluctuating levels [100–102]. Accordingly, VM grafts have been found to reduce the severity of pre-established L-DOPA-induced dyskinesias [103] and to normalize the expression of striatal DA receptors and markers of maladaptive molecular plasticity in 6-OHDA-lesioned rats [103, 104]. One obstacle to the further clinical development of neural transplantation is the occurrence of post-operative dyskinesias, which have been reported in a significant proportion of transplanted human patients (reviewed in [105]). The induction of abnormal involuntary movements by intrastriatal transplants had not been predicted by animal experiments, and the mechanisms underlying this form of dyskinesia are poorly understood. Novel dyskinetic motor patterns in the late post-grafting period are usually independent of the timing of L-DOPA administration, and may even coexist with an improvement of peak-of-dose L-DOPA-induced dyskinesia [105], suggesting that the two forms of treatment-related dyskinesias have distinct mechanisms. The clinical findings have stimulated a reappraisal of the behavioural effects of VM grafts in animal models of PD. Interesting observations have recently emerged from a careful behavioural analysis of 6-OHDA-lesioned rats implanted with embryonic VM tissue in the striatum. In keeping with previous studies, the grafted animals showed a marked post-operative improvement of L-DOPA-induced dyskinesia. They however also developed novel dyskinetic behaviours [106, 107, 108]. Pronounced dyskinesia could be elicited in grafted rats by challenge doses of amphetamine or DA reuptake blockers [106, 107]. This form of graft-related dyskinesia was conditioned by the number of DA neurons in the transplants [107], and by their regional placement within the striatum [106], and it could be blocked by DA receptor antagonists (Lane et al., unpublished observations). Although the mechanisms of graft-induced dyskinesias are presently unclear, this phenomenon underscores that cell-based approaches to CDS offer less predictable outcomes than drug delivery methods.

Adjunct Treatments to Prevent or Treat Motor Complications

One potential approach to prevent or treat motor complications consists in associating non-dopaminergic drugs to standard L-DOPA pharmacotherapy [109]. Compared to CDS, this approach offers the advantages of being easier to administer and probably less expensive. Moreover, some non-dopaminergic treatments may even ameliorate psychiatric or cognitive symptoms that are L-DOPA resistant [110]. At present, such advantages should be seen as mere potentialities, because the clinical evaluation of non-dopaminergic drugs has not yet delivered concrete therapeutic options for PD. In a vast scenario of possible approaches [109, 110], broad interest has been raised by two categories of non-dopaminergic compounds, acting on glutamate and 5-HT receptors, respectively.

Glutamate transmission is involved in L-DOPA-induced dyskinesia at multiple pathophysiological levels (recently reviewed in [94, 110, 111]). Compounds with antagonistic properties at *N*-methyl-D-aspartate (NMDA) [112] or amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors [113] have shown antidyskinetic efficacy in animal experiments and small clinical trials in PD patients [114–116]. As a matter of fact, amantadine, an anti-infectious agent that exerts weak non-competitive antagonism at the NMDA receptor [117], is the only non-dopaminergic drug currently used for the clinical management of L-DOPA-induced dyskinesia [118–121]. Antagonists that selectively interact with the NR2B subunit of NMDA receptors have been shown to prevent the development of dyskinesia in non-human primate models of PD [122]. On a cellular level, the close functional interactions between post-synaptic DA and glutamate receptors on striatal neurons (recently reviewed in [21, 22, 94, 110, 111]) suggest that glutamate receptor antagonists may provide the most effective approach to block molecular and synaptic alterations associated with L-DOPA-induced dyskinesia (reviewed in [94, 110, 111]). In experimental studies, promising results have been recently obtained using antagonists of the metabotropic glutamate receptor type 5 (mGluR5), which is abundantly expressed in striatal neurons and becomes upregulated following chronic dyskinesiogenic treatment with L-DOPA [123, 124]. In the rat model of L-DOPA-induced dyskinesia, mGluR5 antagonists can both prevent the development of abnormal involuntary movements and acutely reduce their severity [125]. These effects are accompanied by a normalization of molecular and neurochemical changes that are closely associated with the movement disorder [125, 126].

Growing experimental evidence implicates the brain 5-HT system in the pathophysiology of L-DOPA-induced dyskinesia. Serotonin receptors are expressed both post-synaptically and presynaptically in striatal neurons, where they modulate signalling pathways downstream of DA receptors [127, 128]. Moreover, serotonin neurons provide the main route of L-DOPA uptake and conversion in the brain when the nigrostriatal DA projection is severely compromised. These neurons lack high-affinity DA reuptake mechanisms and DA autoreceptors in their axon terminals, and therefore represent a source of unregulated DA release into the extracellular fluid (reviewed in [94, 129]). The pathophysiological importance of this phenomenon is illustrated by the fact that coadministration of L-DOPA with agonists of 5-HT1A serotonin autoreceptors blunts the increase in striatal extracellular DA levels induced by L-DOPA [130] and also attenuates the severity of dyskinesia [131–133]. In rats with 6-OHDA lesions, combined treatment with 5-HT1B and 5-HT1A receptor agonists is particularly effective in reducing the severity of L-DOPA-induced dyskinesia, achieving an effect similar in magnitude to that of a lesion of forebrain serotonin projections [129]. Substances with agonistic activity at the 5-HT1A receptor have shown antidyskinetic efficacy in small clinical trials in PD patients [134–136]. Regulatory approval of 5-HT1A receptor agonists for the treatment of dyskinesia will be conditional to the replication of these effects in large-scale trials.

The Challenge of L-DOPA-Resistant Symptoms

A variable extent of autonomic, cognitive and psychiatric dysfunction usually accompanies the motor syndrome of PD [137]. It has become increasingly recognized that these non-motor features represent an important source of disability for PD patients and have a major negative impact on their quality of life [138, 139]. Several non-motor features are poorly responsive to, and may even be worsened by, DA replacement therapy (Table 20.2). The pathophysiology of these deficits is poorly understood. In addition to a dopaminergic component, cognitive, psychiatric and autonomic symptoms are likely to depend on the degeneration of other aminergic and peptidergic systems during the progression of PD [140–142]. Symptoms that are poorly responsive to dopaminergic therapy also include some motor deficits occurring in the advanced stages of PD, such as severe balance problems with falling episodes, and freezing of gait. These symptoms affect greater than 50% of PD patients in late disease stages and can be severely disabling [10, 138].

Cognitive impairments in PD predominantly affect executive functions, such as planning, attentional set-shifting, procedural learning and working memory, where spatial recognition memory is the earliest affected form [143, 144]. Much of these deficits can be attributed to DA denervation of non-motor frontostriatal circuits, which becomes more extensive as the disease progresses [141, 143, 145]. While certain aspects of cognitive function, such as response time, are ameliorated by L-DOPA, other aspects are not improved [146] or may even become further impaired by the treatment. Cognitive functions that are negatively affected by the treatment include reversal learning [147, 148] and the capacity to learn from negative decision outcomes [149], which seems to be impaired by the sustained stimulation of D2/D3 class receptors [150, 151]. A significant problem in advanced PD is the development of overt dementia, which is mainly attributed to widespread neurodegeneration and Lewy body pathology affecting also the cerebral cortex [140]. The cumulative incidence of dementia steadily increases with age and duration of PD, reaching rates as high as 80–90% by 90 years of age [152].

In the psychiatric domain, L-DOPA treatment generally improves motivation and reduces fatigue and anxiety [148], thus raising the patients' general sense of well-being. The combination of DA denervation and dopaminergic

Table 20.2 Symptoms poorly responsive to dopaminergic therapy in PD

Motor	Dysarthria, balance impairment and falls, freezing of gait
Sensory	Olfactory Dysfunction, pair
Autonomic	Orthostatic hypotension, neurogenic bladder with urinary frequency–urgency–incontinence, constipation, sexual dysfunction, abnormal sweating and salivation
Cognitive	Negative feedback learning*, attentional set-shifting, dementia
Psychiatric	Depression, sleep problems (poor sleep quality at night and abnormal daytime somnolence)*, hallucinations*, impulse control disorders*

Asterisks mark the symptoms that are aggravated or induced by dopaminergic medications.

therapy can, however, also induce a range of complex behavioural disorders, which are not yet completely defined. The so-called "dopamine dysregulation syndrome" is a medication-induced state originally described in young PD patients who self-administered L-DOPA at a dosage much above that required to control their motor symptoms [153, 154]. This abnormal drug usage was associated with hypomanic/manic mood and behavioural features causing significant social impairment (e.g. fights, arguments, absence from work). Partial withdrawal from medications leads to a negative affective state (dysphoria, depression, anxiety [153, 154]). The term "impulse control disorders" includes a range of behavioural abnormalities, such as pathologic gambling, compulsive shopping, pathologic eating, hypersexuality and punding (compulsive performance of repetitive tasks). These are particularly common in patients treated with DA agonists [150, 153, 155]. The life time prevalence of impulse control phenomena has been estimated to be about 6% in the general PD population and 14% in PD patients treated with DA agonists [156].

Clinically relevant depressive symptoms affect approximately 35–40% of PD patients [157–159]. This high incidence is likely to depend on specific neurobiological derangements, and not on a generic psychological reaction to the disease [25, 137]. Moreover, the pharmacotherapy may cause mood swings. In the complicated stages of PD, motor fluctuations are paralleled by distressful mood fluctuations, where the L-DOPA "off" periods can be accompanied by aphasia, desolation, poor emotional control and slowness of thinking [25, 160].

Sleep disturbances are common in PD, their prevalence ranging between 60 and 98% in different studies [161]. Sleep problems may occur early and even precede the diagnosis, but are generally more frequent and more severe in patients with advanced PD [162]. The causative factors are multiple and include depression, neurodegenerative processes in brain stem nuclei that control sleep and side effects of the medications [161–163]. Among the most common sleep disturbances are REM sleep behaviour disorder (RBD), insomnia, nightmares, snoring, restless legs and sleep ambulism. Daytime sleepiness is common and can be aggravated by the therapy [161].

Autonomic dysfunction is very common in PD, and it is attributed to an involvement of the central and peripheral autonomic nervous system in the pathological process, which worsens as the disease progresses [10, 164]. Dysfunction can occur at many levels (gastrointestinal, urogenital, cardiovascular, sudomotor and thermoregulatory). The most common symptoms consist in constipation, urinary incontinence, orthostatic hypotension and heat/cold intolerance [164, 165]. Orthostatic hypotension has a prevalence of 15–20% and can be worsened by dopaminergic treatment. Urinary disturbance (most commonly urinary frequency, urgency and nocturia) occurs in 27–39% of PD patients [164, 165]. Sweating disturbance (hypo- as well as hyperhidrosis) occurs in a majority of patients. Changes in sexual interest and functions are very common, and the dopaminergic treatment can have stimulating as well as suppressing effects on libido. It has been recently recognized that hypersexuality may occur as a side effect of dopaminergic treatment (see impulse control disorders above).

Current Treatment Options for L-DOPA-Resistant Symptoms

Cognitive Dysfunction

Several studies have suggested that cholinesterase inhibitors (such as tacrine, donepezil, rivastigmine and galantamine) can be beneficial in the treatment of dementia in PD [164]. Most studies in this area have been performed using an open-label design and small sample size. For rivastigmine, however, a large-scale double-blind study has demonstrated significant improvements of global ratings of dementia, cognition and behavioural symptoms among Parkinson patients with dementia [166, 167]. Tremor was seen as a side effect and led to termination of therapy in 1.7% of the rivastigmine-treated patients. Furthermore, gastrointestinal side effects were relatively common [166, 167]. The latter problem may largely be overcome by using the rivastigmine patch preparation, which has recently become available [168].

Dopamine Dysregulation Syndrome and Impulse Control Disorders

At present, there is a fundamental lack of studies regarding the management of dopamine dysregulation syndrome and impulse control disorders. Since impulse control phenomena are more common in DA agonist-treated patients, a first step to their management should consist in discontinuing the DA agonists, as suggested by one recent study [169]. Antiandrogens may provide an effective option for hypersexuality. Several centres have also observed that CDS can have advantages with respect to both dopamine dysregulation syndrome and impulse control disorders (unpublished data from Odin and collaborators).

Psychosis

The risk for psychosis seems to be higher if several Parkinson drugs are combined. For this reason, when psychotic symptoms appear, the first step to their treatment consists in reducing the number of drugs [164]. Drugs with a high risk–benefit ratio of psychotic symptoms versus antiparkinson effect are reduced first (thus, anticholinergics and amantadine before DA agonists and levodopa). A compromise between motor function and mental state has to be reached. Treatment with cholinesterase inhibitors might be helpful [164]. Among the antipsychotic drugs, the atypical neuroleptic, clozapine (6.25–100 mg daily), is the only medication shown to improve psychosis without worsening PD motor symptoms [170].

Depression

Several tricyclic antidepressant drugs have shown efficacy in the treatment of depression in PD in randomized, controlled, double-blind trials [164]. Because of their side effect spectrum (cognitive side effects, in particular), these drugs are, however, seldom prescribed in PD. Selective serotonin reuptake inhibitors (SSRI) are probably the most widely used class of medications to treat depression in Parkinson patients, but very few controlled drug trials have been reported in this field [171, 172]. Data from PET investigations would suggest that noradrenergic mechanisms may be even more important than serotonergic ones in parkinsonian depression [173]. This would suggest that medications increasing noradrenaline levels may be particularly beneficial to treat depression associated with PD, and recent studies are lending support to this prediction [174].

Sleep

The first step to treat sleep disorders in PD should be a thorough analysis regarding the type of disturbance and the possible underlying reasons [162]. If sleep problems are caused by night-time Parkinson symptoms, addition of long-acting dopaminergic drugs in the late evening could prove useful. Long-acting DA agonists may also improve restless legs symptomatology. On the other hand, reduced evening dosage of dopaminergic medications is indicated when dopaminergic drugs seem to disturb sleep. Other options to treat sleep problems in PD consist in drugs that have been successfully used for this indication in elderly people, which include some antidepressive drugs acting at 5-HT or noradrenergic receptors, as well as benzodiazepines [175, 176]. In the latter category, clonazepam has the best documented effect against REM sleep behaviour disorder [177].

Autonomic Symptoms

Very few treatments have been tested to ameliorate autonomic symptoms in PD. One of the few exceptions is a randomized, controlled trial with sildenafil (Viagra) showing efficacy in male patients with erectile dysfunction [178]. Another interesting study concerns the positive effects of an isosmotic macrogol electrolyte solution for the treatment of constipation [179].

L-DOPA-Resistant Gait, Freezing and Balance Problems

Physical therapy methods aimed at training protective postural responses have proven useful in patients affected by gait and postural instability [180, 181].

Moreover, recent data suggest that electrical stimulation of brain stem structures involved in the control of gait and posture, such as the pedunculopontine nucleus, can improve gait and balance problems that are resistant to other treatments [182].

Concluding Remarks

Based on the body of knowledge generated by experimental and clinical studies, it is realistic to foresee significant improvements in DA replacement therapy for PD during the coming few years. Some forms of CDS, such as subcutaneous apomorphine infusion and enteric L-DOPA delivery, have already been proven as a viable therapeutic option for patients in the complicated stages of PD. Observations from patients receiving continuous L-DOPA or apomorphine treatment suggest that CDS can improve not only motor complications, but also some of the non-motor symptoms of PD (e.g. non-motor off periods and sleep problems). Clinical studies to verify these observations are ongoing in several centres. Moreover, approaches to CDS based on gene therapy are being explored in animal models of PD with successful results. Eventually, it will be the balance between efficacy, safety and costs to determine which of the new potential CDS options will turn into an acceptable treatment. In addition to CDS, adjunct pharmacological treatments to prevent or treat motor complications represent a realistic possibility. Here the clues provided by animal studies are already multiple and very promising, and the translational bottleneck is represented by the difficulty to verify all these clues in appropriately designed clinical trials. For example, it is still a matter of debate which assessment methods provide sufficient sensitivity and reliability to test antidyskinetic treatments in human studies [183].

During the last few years we have become increasingly aware of the fact that PD does not solely affect movement and that non-motor features may represent a significant burden for the patients. At the same time there is a dramatic lack of good-quality trials regarding treatment of non-motor symptomatology. Along with disease-modifying interventions, treatments for cognitive, psychiatric and autonomic features represent urgent unmet needs in the therapy of PD. More extensive pathophysiological investigations in patients, associated with symptomatic modelling in animals, will be required for pharmacological research to advance at a faster pace in this complex area.

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Chapter 21
Molecular, Cellular and Electrophysiological Changes Triggered by High-Frequency Stimulation of the Subthalamic Nucleus in Animal Models of Parkinson's Disease

Paolo Gubellini and Pascal Salin

Introduction

Deep brain stimulation (DBS) at high frequency, thus also called high-frequency stimulation (HFS), is now widely utilized as a surgical strategy for the treatment of several movement disorders as well as pain, depression and obsessive-compulsive disorder. Regarding movement disorders, HFS of the subthalamic nucleus (STN) or the internal segment of the *globus pallidus* (GPi) is highly effective in reducing the symptoms of Parkinson's disease (PD) including akinesia, tremor, rigidity and gait impairments. Moreover, stimulation of the ventral intermediate nucleus (VIM) of the thalamus gives great benefit to patients with limb tremor, while dystonia is ameliorated by stimulating the GP. However, despite the undoubted therapeutic efficacy of DBS, there are still several controversies about its mechanisms of action. In addition, although the clinical advantages of DBS are undisputable compared to surgical lesions, its possible consequences on brain circuitry and function are still under debate. To address these issues experimentally, several research teams have been studying the effects of DBS in animal models. In this chapter, we will focus on the vast domain regarding the effects of DBS in animal models of PD at molecular, cellular and neurophysiological level. A brief introduction on the history of this treatment, on its clinical relevance and on the PD models utilized in these studies will provide the basis for this review.

Deep Brain Stimulation and Parkinson's Disease

Electrical stimulation assisted by stereotaxy was developed in the late 1940s in order to help identify and map deep brain structures [1]. Since the 1950s, DBS

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has been utilized for intrasurgical localization prior to therapeutic lesion of several brain structures, in particular the GP and the thalamus [2]. It was only in the 1960s that HFS of the ventrolateral thalamus was reported to alleviate tremor [3, 4]. However, during this decade DBS was still utilized for targeting a brain region prior to its lesion: in some cases, DBS was delivered with chronically implanted electrodes over a period that could reach several days, in order to best define the target to be lesioned. In the 1970s and 1980s, the therapeutic use of chronic stimulation of the cerebellum emerged for treating movement disorders or epilepsy [5–7] and thalamic DBS for alleviating pain [8]. In particular, Benabid and collaborators [9] reported in 1987 that stimulating the VIM could ameliorate PD tremor during the targeting procedure for surgical lesioning this structure. Such observation led to the application of VIM chronic stimulation for the treatment of PD, essential tremor and extrapyramidal dyskinesias, which was the first example of DBS delivered by chronically implanted electrodes connected to a pacemaker-like portable stimulator [10]. Since then, this surgical technique, applied to several brain structures, has become widespread among the treatment of a variety of brain disorders [11].

The rationale for targeting STN in the treatment of PD came from the knowledge that surgical lesion of this structure has beneficial effect on parkinsonian motor symptoms. However, the first experimental evidence that HFS could replace lesion came from a study in the monkey by Benazzouz and colleagues in 1993 [12], showing that this technique could alleviate parkinsonian rigidity and bradykinesia, without causing dyskinesia or hemiballismus as after STN lesion. In line with this finding, the technique was successfully applied to parkinsonian patients [13]. The therapeutic frequency range for bilateral STN HFS that significantly reduces the classical PD motor symptoms (including tremor, bradykinesia and gait impairment) is between 80 and 185 Hz [14–21]. Targeting the STN by HFS also provides the great bonus consisting of the possibility of reducing dopaminergic medication: this is a considerable advantage since also drug-induced dyskinesia is reduced, even if indirectly [15, 22–24]. The benefits provided by STN HFS, however, do not exceed those of levodopa therapy, measured according to the Unified Parkinson's Disease Rating Scale (UPDRS: multiple ratings that measure motor function and also mental functioning, behaviour, mood and activities of daily living [25, 26]); the improvement of some executive functions has also been reported during STN HFS [27]. Moreover, DBS presents another interesting advantage over levodopa treatment: it reduces and even eliminates the *off* state time, during which the patient is prostrate with rigidity and bradykinesia. While patients are usually implanted bilaterally, there is now increasing evidence that unilateral STN HFS is also effective in reducing motor symptoms during the *off* medication state [28–32]. However, although unilateral stimulation reduces the risks of a bilateral surgery, it can be challenging when this treatment is coupled to

levodopa, because dyskinesias can be induced asymmetrically by HFS itself and/or by levodopa that acts bilaterally. Thus, bilateral stimulation is still preferred to unilateral. Besides the above-described positive effects on motor performance, however, STN stimulation can have undesired side-effects and drawbacks. It has been shown that it can reduce working memory and impair cognitive motor control [33] as well as conditional associative learning [27]. In a few cases, STN DBS can produce psychiatric effects including cognitive alterations, hallucinations, manic episodes, mood disorders and impulsive behaviour [11, 34]. During the last decade, several studies performed in parkinsonian patients during DBS electrode implantation have revealed, by measuring local field potential, that basal ganglia(BG)–cortical loops have an oscillatory and synchronized activity between 8 and 30 Hz, known as the "beta frequency band". Interestingly, such synchronization is disrupted by levodopa treatment and STN HFS, suggesting that (i) beta oscillations of BG–cortical loops might be related to PD motor impairment and (ii) one action mechanism of DBS, as well as dopaminergic medication, could be the disruption of this synchronization [35–37]. Although STN represents the main and probably the best target for the treatment of PD by DBS, other brain structures have been also successfully targeted, such as GPi, VIM and, more recently, pedunculopontine nucleus (PPN) and centre median-parafascicular (CM/Pf) complex of the thalamus.

Bilateral HFS of the GPi reduces all main motor manifestations of PD [38]. However, its efficacy depends on the modality and site of stimulation, and GPi stimulation has reported having questionable effects on extrapyramidal signs and worsening conditional associative learning. Moreover, chronic HFS of the GPi does not allow reducing levodopa treatment, thus the side-effects associated to the use of this drug persist after electrode surgery [27, 39–43].

Stimulation of the thalamus in the VIM region is able to reduce limb tremor but has little or no effect on the other motor symptoms of PD, in particular bradykinesia and rigidity. Moreover, in some cases VIM DBS loses its efficacy after a few years and parkinsonian patients under thalamic stimulation still need dopaminergic medication [44–46].

Recently, the PPN has been proposed as a possible target for DBS [47, 48]. Stimulating PPN (at "low" frequencies around 20–25 Hz) is beneficial for reducing parkinsonian motor troubles, but its effect on hypokinetic signs is not impressive, as well as its effect during *off* levodopa medication state [49]. However, what seems potentially interesting is that PPN stimulation combined with STN HFS strikingly ameliorates UPRDS score during the *on* time. This evidence might support the selection of previously STN-implanted patients with poor therapeutic response for additional PPN targeting. That said, the unimpressive effect of PPN DBS alone suggests that PPN is not a fully alternative target [49, 50].

Finally, HFS of the CM/Pf has been recently performed in PD patients with significant motor therapeutic effects [51]. CM/PF DBS is very effective in

reducing tremor, indicating this complex as a promising target in advanced PD patients.

Experimental Models of Parkinson's Disease

The discovery of drastic striatal dopamine depletion in patients with PD and the development of antiparkinsonian drug therapy were largely based on animal models. During these past 50 years, several different animal models have been developed to study this neurodegenerative movement disorder. Although acute models of PD were first introduced by using monoamine-depleting agents, such as reserpine, and later by using dopamine receptor antagonists, such as haloperidol, the most commonly used PD animal models are actually based on toxin models, using 6-hydroxydopamine (6-OHDA) in rodent and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in non-human primates and mouse. Here is a brief review of the main experimental PD models.

Reserpine

The use of reserpine was one of the first rodent models of PD. The starting point for its discovery was the observations by Carlsson and colleagues, in 1957, that a depletion of dopamine from the striatum is responsible for the motor symptoms of PD and that an akinetic state could be elicited in rats by systemic administration of reserpine, and finally that this state could be alleviated by dopaminergic treatments with L-DOPA [52]. Reserpine injection induces a reduction in motor activity (akinesia, hypokinesia, catalepsy) associated with a depletion of monoamine intraneuronal storage vesicles, via magnesium- and ATP-dependent mechanisms. However, the monoamine depletion induced by this treatment is transient and non-selective (adrenaline, noradrenaline, histamine, serotonin, etc.). Moreover, reserpine administration does not provoke concomitant cell death in mesencephalic dopamine structures.

Haldol/Haloperidol

Classic neuroleptics such as haloperidol, as dopamine D2-receptor antagonists, give rise to effects which are similar to those occurring after reserpine treatment. The administration of haloperidol at low doses (0.15 or 0.3 mg/kg i.p.) may also result in a behavioural state resembling the symptoms of akinesia and bradykinesia in PD [53]. At higher doses (0.5–1 mg/kg i.p.) haldol injection induced catalepsy, defined as the delayed or absent correction of an abnormal positioning of the forelimbs. This behavioural effect is due, in part, to dopamine striatal transmission blockade on both D1 and D2 receptors. This pharmacological treatment does not induce dopaminergic cell loss.

6-OHDA

Since its first description in 1959, the neurotoxin 6-OHDA has played a fundamental role in research on PD. 6-OHDA is a structural analogue of catecholamines and exerts its toxic effects on catecholaminergic neurons. Neurotoxic effects of 6-OHDA occur through a two-step mechanism involving accumulation of the toxin into catecholaminergic neurons, followed by alteration of cellular homeostasis and neuronal damage. Intracellular storage of 6-OHDA is mediated by the dopamine or noradrenaline membrane transporters. 6-OHDA is toxic at both a peripheral and central level. However, since this molecule is unable to cross the blood–brain barrier, toxicity in the CNS is achieved only when the 6-OHDA is directly injected into the brain by stereotaxic procedure. The dopaminergic selectivity of 6-OHDA is done by targeting the dopaminergic structures in association with a noradrenergic uptake blocker treatment, such as desipramine. 6-OHDA injection at dopamine neuronal level in the substantia nigra pars compacta (SNc) or at dopaminergic terminal level in the striatum, or again in the medial forebrain bundle (fibre level), produces a degeneration of dopaminergic neurons and specific physiological and motor deficits, which mimic those of human PD.

MPTP

Another experimental model of PD is the so-called MPTP model. MPTP is a mitochondrial complex I inhibitor that was accidentally discovered in drug-dependent Californians who had injected a new "synthetic heroin" [54, 55]. The potential of this substance to produce the Parkinson's syndrome was subsequently confirmed in various animal families and particularly in non-human primates. Highly lipophilic, MPTP easily crosses the blood–brain barrier and can be administrated systemically. It becomes toxic through its transformation to MPP^+ by the enzyme MAO-B present in glia and serotoninergic neurons. The dopamine transporter system then transports MPP^+ into dopaminergic neurons. Inside neurons, MPP^+ impairs oxidative phosphorylation by inhibiting the enzyme complex I of the mitochondrial electron transport chain [56] causing a rapid and profound depletion of cellular ATP levels. Various conditions of MPTP administration (acute vs. chronic; repeated injections of low doses vs. a single high dose) exist in the literature. The toxicity of MPTP in monkeys mimics best the PD symptomatology in human patients while the rat, but not the mouse, appears to be refractory to MPTP.

Electrophysiological Properties of STN Neurons

The electrophysiological and membrane properties of STN neurons have long been characterized by several groups [57–62]. These cells are spontaneously

active and fire action potentials (APs) at the resting membrane potential (RMP) or when they are depolarized. The frequency of APs at RMP is variable, ranging from less than 10 Hz up to 20–25 Hz, depending on the recording condition and on the RMP value. When depolarized by strong current injection, these cells can reach spike frequencies up to 300–500 Hz. Approximately half of the STN neurons have a spontaneous and tonic firing activity, also called "single-spike" mode (Fig. 21.1A). The other half can switch from tonic to burst-like firing pattern, or "burst" mode. This switch is voltage dependent, the burst mode being triggered by hyperpolarizing the neurons at about −42 to −60 mV, while at potentials around −35 to −50 mV the firing pattern reverses to single spike. At membrane potentials more hyperpolarized than −60 to −70 mV most STN neurons are silent (Fig. 21.1B). Two types of burst firing patterns have been observed in STN neurons: a "pure" burst mode consisting of regular burst firing episodes separated by short quiescent periods and a "mixed" burst mode consisting of long burst separated by sequences of short bursts (Fig. 21.1C). Other authors have classified these three AP firing types as "normal" or "tonic", "bursting" or "phasic" and "mixed" or "phasic-tonic", respectively [63, 64]. Single-spike activity (Fig. 21.1A) of STN neurons is a spontaneous peace-maker-like activity, which is insensitive to antagonists of ionotropic glutamate receptors and $GABA_A$ receptors, blockers of Ca^{2+} currents (such as Co^{2+} or Ni^{2+}) or intracellular Ca^{2+} chelators (such as BAPTA). This means that it is independent from synaptic afferences and Ca^{2+} or Ca^{2+}-activated currents. Single-spike activity mainly results from two depolarizing cationic currents: the so-called persistent Na^+ current (I_{NaP}) that activates below spike threshold and inactivates slowly, and the hyperpolarization-activated cation current (I_h), which contributes in maintaining membrane potential at depolarized values [57, 61, 62]. Burst mode activity (Fig. 21.1C) results from phases of cyclic and alternate activation/inactivation of depolarizing and hyperpolarizing currents. The phases are illustrated in Fig. 21.2: depolarization (b–c), slowly decaying plateau (c–d) and repolarization to the after-hyperpolarizing potential (d–a), corresponding to the most negative potential recorded in a given neuron (the RMP of spontaneously firing neurons is difficult to establish because of the absence of a stable membrane potential). The voltage dependence of the currents implicated in the generation of this activity is presumably also responsible for the switch between the firing modes (single-spike to burst mode) of STN neurons [60]. However, also synaptic mechanisms are implicated in the regulation of STN neurons activity. Bevan and colleagues [65] have shown that GABAergic input from the GP interplays with membrane properties of STN neurons to produce different patterns of firing.

In animal models of PD, several changes in STN neuron firing activity and pattern have been observed in vivo. In rats with 6-OHDA injection in the SNc, the observed changes seem to be contradictory: Kreiss and colleagues [66] have shown an increase in the frequency of spontaneous spikes associated with a more regular firing pattern and a smaller percentage of cells with a pattern of bursting activity. Hassani, Vila and colleagues [67, 68] have also shown

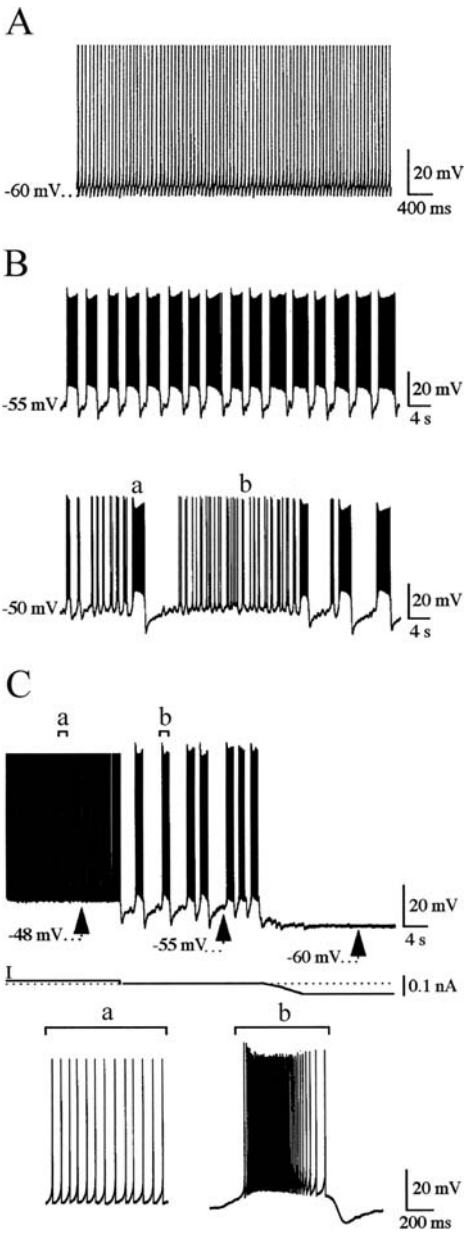

Fig. 21.1 Spontaneous firing properties of STN neurons recorded in vitro. (**A**) Tonic and regular activity of a STN neuron: single-spike mode (mean inter-spike interval 66.1 ± 15.6 ms). (**B**) Two types of burst mode: pure burst mode (*upper trace*) and mixed burst mode (lower trace) consisting of long bursts (a) separated by sequences of short bursts (b). (**C**) Firing mode switches from single-spike (a) to pure burst mode (b) by hyperpolarizing the neuron from −48 to −55 mV. At more hyperpolarized potentials (−60 mV) all spontaneous firing activity is suppressed (modified from [60])

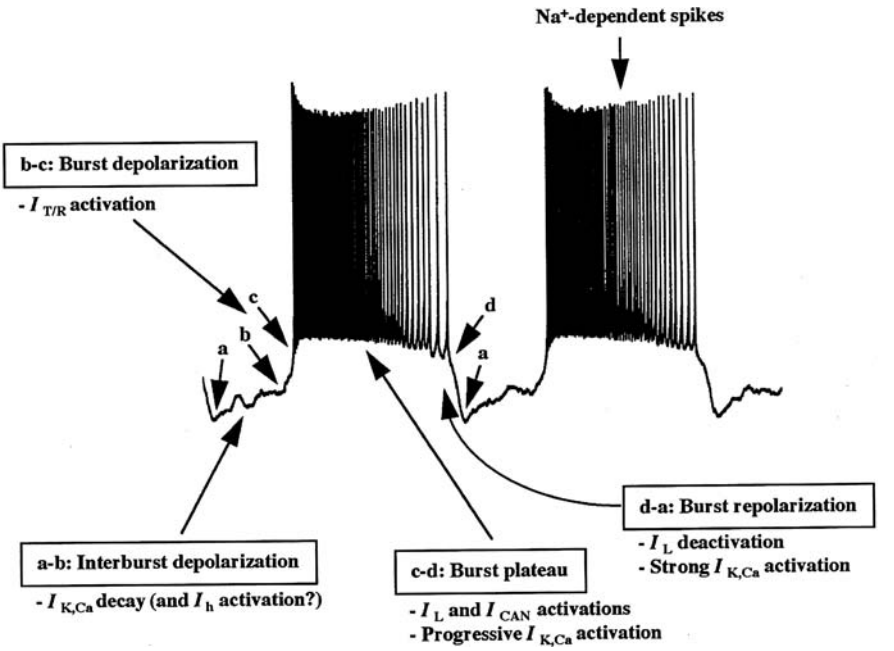

Fig. 21.2 The hypothetical cascade of currents underlying the different phases of burst firing mode. The depolarization phase (b–c) results from a low-threshold T/R-type Ca^{2+} current ($I_{T/R}$) that depolarizes the membrane to the threshold potential of L-type Ca^{2+} current (I_L) and then inactivates. The slowly inactivating I_L depolarizes the membrane to the plateau phase, during which spikes are evoked (c–d). Spikes amplify Ca^{2+} entry by activating more I_L and, possibly, other types of high-threshold Ca^{2+} currents (such as the non-specific cationic current, I_{CAN}). The resulting increase in intracellular Ca^{2+} concentration activates the Ca^{2+}-activated K^+ currents ($I_{K,Ca}$). The balance between depolarizing (I_L) and hyperpolarizing ($I_{K,Ca}$) currents, slightly in favour of the latter, explains the gradual decline of the plateau towards the repolarization phase. When membrane potential has declined to a certain level, it suddenly repolarizes (d–a) because of rapid I_L deactivation and stronger $I_{K,Ca}$ activation. This leads to the peak of after-hyperpolarizing potential, during which $I_{T/R}$ activates, leading to a new membrane depolarization (a–b) as $I_{K,Ca}$ decays because of Ca^{2+} clearance mechanisms. The depolarization to the threshold potential of $I_{T/R}$ thus initiates a new cycle of the burst mode. A hyperpolarization-activated cation current (I_h) may also participate in the slow depolarization (a–b) between two consecutive bursts (from [60])

increased spike frequency of STN neurons, but a more irregular firing pattern with bursts. Other groups also reported an increase in the proportion of STN neurons showing a burst firing pattern, but they observed no significant changes on spike frequency [63, 69–71]. In monkeys treated by MPTP, the spontaneous firing rate is slightly increased and the percentage of neurons showing burst activity is also enhanced. Periodic oscillatory neuronal activity at low frequency, which in humans is highly correlated with tremor, has also been detected in a large proportion of STN neurons after MPTP treatment

[72–74]. Similar oscillatory patterns in the beta frequency range have also been recorded in parkinsonian patients and are thus presumed to be associated to the disease and the consequent limb tremor [75–86]. Unfortunately, while in vivo PD models allowed the assessment of these dramatic changes in STN neuron activity, in vitro preparations failed, to date, to reveal similar modifications.

High-Frequency Stimulation of the STN: In Vitro Approaches

The observation that the surgical destruction of the STN ameliorates parkinsonian motor symptoms suggested that STN HFS may act by blocking the electrical activity of this structure, thus functionally acting itself as a lesion. To study how STN HFS affects the intrinsic activities of STN neurons, in vitro slice preparations are a very good option because they enable to better isolate the effects of a tetanic stimulation on neuronal properties. Thus, it is not surprising that the majority of in vitro data concerning the effect of STN HFS have been provided by electrophysiological slice recordings. On the other hand, most of the experimental evidences on STN HFS obtained utilizing these in vitro approaches have focussed on the STN itself, although a critical question regarding DBS deals with its effect not only on the target structure but also on brain circuitry and function of connected and surrounding structures. The main reason of this was that no brain slice preparation allowed the preservation of the whole BG connectivity, at least not until Beurrier and collaborators developed the "basal ganglia slice", in which the interconnectivity between the GP and the STN and the projection of the STN to the SN are preserved and functional [87]. Unfortunately, to date a study on STN HFS utilizing this preparation is not yet available. A small subset of in vitro studies deals with measuring neurotransmitter release during STN HFS, in particular of glutamate and GABA that are the main neurotransmitters of the BG. It should be mentioned that, to date, all but one of the existing in vitro studies have been performed on slices from control animals rather than from PD models. While this seems in contrast with the topic of this chapter, these studies are worth mentioning since they have provided a valuable contribution for better characterizing important and fundamental effects of STN HFS.

Electrophysiological Effects of In Vitro STN HFS

The first report showing in vitro the effect of STN HFS was provided by Beurrier et al. [88], who preformed electrophysiological patch-clamp recordings from slices of 3- to 4-week-old rats. The stimulating electrode was placed in the

middle of the STN. Two types of electrodes were used: a 0.5 mm diameter bipolar concentric electrode or a much thinner one (0.01 mm) designed to minimize tissue damage. HFS consisted of trains lasting 1 min, with 100 μs wide stimuli of 5–8 V amplitude, at frequencies ranging from 100 to 500 Hz. Interestingly and in agreement with the hypothesis that DBS should inhibit the activity of the STN, HFS blocks the spontaneous firing activity of STN neurons. This effect is clearly frequency dependent, because at frequencies of 166–250 Hz the blockade is total, but at 100–125 Hz some spike activity is spared (Fig. 21.3). The inhibitory effect is the same in neurons with single-spike activity, and in those with bursting activity, it is reversible (spike activity recovers after ~6 min), and HFS can be repeated several times with the same effect, as long as the recording lasts. The inhibition, as depicted in Fig. 21.3, occurs after HFS has been stopped. The basic mechanism by which HFS silences STN neurons seems to be exclusively mediated by a dramatic reduction of Na^+ and Ca^{2+} voltage-gated currents, leading to the disruption of spontaneous firing activity. The blockade of APs does not depend upon transient membrane hyperpolarization, since membrane potential of the depressed neurons is more depolarized compared to depolarized potentials at which cells were silent prior to HFS (see Fig. 21.1B). Moreover, the suppression of STN neurons firing activity is also clearly independent from synaptic neurotransmitter release: the application of $GABA_A$, AMPA and NMDA receptor antagonists, as well as the blockade of synaptic transmission (by perfusing the slice with Co^{2+}-containing solution), does not alter the effect of HFS. Accordingly, an inhibitory effect similar to that provided by extracellular HFS can be obtained by intracellular HFS (i.e. delivering depolarizing pulses through the recording electrode), confirming that spike activity blockade does not result from the activation of a local network and it is not mediated by the stimulation of STN afferents. However, while the post-HFS effect has been clearly shown by this

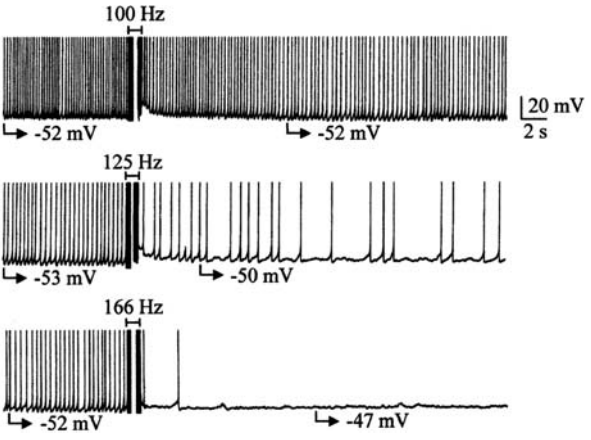

Fig. 21.3 Post-effect of STN HFS on the firing activity of STN neurons. The traces are from the same STN neuron with single-spike firing mode. Applied at 100 Hz (*upper trace*), HFS has nearly no post-effect, whereas at 125 Hz (*middle trace*) it decreases the frequency of tonic activity and at 166 Hz (*lower trace*) stops single-spike activity for 5 min (from [88])

study, what happens during HFS still needed to be characterized; the major problem to solve was getting rid of the stimulation artefacts occurring during HFS. It was in 2002 that Magariños-Ascone and collaborators showed what is actually happening during HFS, using slices from 9- to 14-day-old rats (stimulation parameters: 100–130 Hz, 0.2–1.0 μA, lasting 40–60 s, delivered by a 80 μm diameter bipolar electrode) [64]. Intracellular recordings show that a steady membrane depolarization is induced by HFS: this depolarization initially triggers AP failures, followed by high-frequency spike activity during the first 5–10 s of HFS and then by burst activity until the neuron is silenced after 15–20 s of stimulation. At lower frequencies (70–90 Hz), HFS also induces a stable depolarization with initial AP failures, followed in a few seconds by a persistent burst firing lasting until the end of the stimulation. A similar increase in AP firing followed by inhibition was also reported by Lee and colleagues in rat slices, where STN was stimulated at 100–140 Hz (for 100–2000 ms at amplitudes of 10–500 μA), and a total AP blockade was obtained at 200 Hz [89]. The same group also showed that STN HFS can trigger excitatory postsynaptic potentials (EPSPs) and increase AP frequency, or vice versa trigger inhibitory postsynaptic potentials (IPSPs) and decrease AP frequency, and that these effects are blocked by GABA and glutamate receptor antagonist [90]. This is surprisingly in contrast with the above findings by Beurrier et al. [88], thus a possible role of synaptic inputs to STN cannot be completely excluded to explain the effects of HFS.

The works by Garcia and colleagues [91, 92] were the first showing the effects of longer stimulation time (30 min to 2 h) on STN neurons activity as well as the first recorded in slices from an animal PD model. More precisely, the model was an acute dopamine depletion one, since it consisted of i.p. injection of reserpine and α-methyl-*p*-tyrosine (a tyrosine hydroxylase inhibitor) in rats, respectively, 20 and 4 h before slice preparation. This was due to the necessity of keeping the age of the animals within 21–23 days to facilitate the patch-clamp electrophysiological recordings. However, this model is hardly comparable to a SNc lesion PD model, especially in terms of functional rearrangements of BG after dopamine depletion. Accordingly, Garcia et al. [91, 92] did not detect any of the known effects observed in vivo on the firing activity of STN neurons after SNc lesion in rats [63, 69, 70]. Not surprisingly, also the effects of STN HFS observed by Garcia et al. [91, 92] were similar in control and dopamine-depleted slices. The stimulating electrode utilized in this group was a monopolar one, with a surface of 0.3 mm^2, and the stimulation consisted of negative current pulses of 60–200 μs and the intensity ranged between 100 and 1500 μA (0.5–3 V). Different frequencies ranging from 10 to 185 Hz were tested in order to compare the effect of those that are relevant to clinical treatment (80–185 Hz) with lower ones. During HFS, both spontaneous spikes and stimulus-evoked APs are present, the former preceded by a depolarization, the latter by an artefact. At 10 Hz, HFS is unable to affect STN neurons activity even at high stimulation intensity,

and at 30 Hz only some cells show a reduction of spontaneous activity. At 50 and 80 Hz, HFS always inhibits STN neuron spontaneous firing. However, these cells are not silent, since their spontaneous activity is completely replaced by a stimulation-driven one (in single-spike or burst mode) at the same frequency of stimulation. Stimulation at 130 or 185 Hz has similar effects, but the frequency of evoked spikes cannot follow that of HFS (especially at 185 Hz): for example, during bursts it is around 64–84 Hz. When HFS is turned off, all STN neurons become silent for a period variable from 20 s to 8 min, similarly to what was described previously by Beurrier et al. [88]. Thus, only HFS delivered at therapeutic range frequency (80–185 Hz) is able to replace spontaneous activity by a stimulus-driven one. See Fig. 21.4 for an example of such HFS-induced activity. However, the parameters of pulse duration (90–200 μs) and intensity (500–800 μA) were adjusted to obtain an electrophysiological response rather than to stick to clinical relevance. This "activity-driving" effect of STN HFS seems to rely on a postsynaptic mechanism involving the activation of Na^+ channels as well as nifedipine-sensitive L-type Ca^{2+} channels. This hypothesis is further supported by the fact that the pharmacological blockade of glutamatergic, GABAergic and aminergic synaptic transmission does not prevent HFS to exert its effects. Overall, these works showing STN neurons activity during HFS [64, 91, 92] support the concept that DBS disrupts the abnormal synchronized oscillatory activity recorded

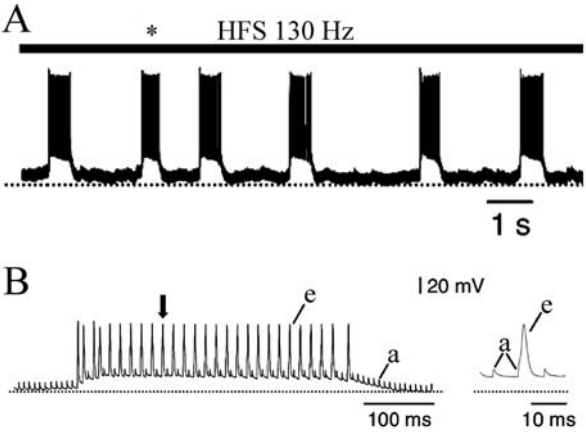

Fig. 21.4 Firing activity of STN neurons during STN HFS. (**A**) Electrophysiological recording of burst activity of a rat STN neuron during HFS at 130 Hz (pulse duration 90 μs, current intensity 550 μA) applied continuously for 30 min. (**B**) On the left, the expanded trace of the burst marked with * in A, showing evoked spikes (e) and stimulation artefacts (a). Note that during the burst the frequency of the evoked spikes is roughly half of that of artefacts, i.e. not all stimuli can trigger an action potential. The spike indicated by the arrow is expanded on the right. Note the two artefacts (a), one preceding and the other triggering the spike (modified from [36])

in the BG-thalamo-cortical loops in parkinsonian state [37], but only when delivered at therapeutic parameters that are able to suppress burst activity and impose a tonic pattern.

Besides the effects of STN HFS on the firing activity of STN neurons, another interesting issue was to characterize how this treatment affects glutamatergic synaptic transmission within this structure. Shen and collaborators [93] addressed this question by stimulating the rostral STN and recording the evoked excitatory postsynaptic currents (EPSCs) from STN neurons by patch-clamp technique. HFS protocol consisted of 60 trains of constant current stimulation at 500 ms intervals, each train containing 50 stimuli (0.1 ms, 50–200 μA) and being delivered at 100 Hz (thus, the entire stimulation protocol lasted 60 s). Three different forms of synaptic plasticity were obtained after this HFS: (1) about 9% of STN neurons showed a short-term potentiation (STP) of the glutamatergic EPSCs evoked by STN stimulation, associated to a decrease in paired-pulse ratio (PPR); (2) about 17% showed a long-term potentiation (LTP) without changes in PPR; (3) about 11% showed a long-term depression (LTD) associated to an increase in PPR. The authors thus suggested that STP and LTD were due to changes in glutamate release from presynaptic terminals: a transient increase for the former, a decrease for the latter. Conversely, LTP maintenance should more likely depend upon postsynaptic mechanisms. This work is the first showing plastic changes evoked by STN HFS at glutamatergic synapses on STN neurons and brings several intriguing findings. In fact, while HFS-induced LTD is consistent with the "classical" inhibitory action of DBS on the excitatory output from the STN, HFS-induced LTP is more consistent with an excitatory action of DBS, as shown by several studies that will be presented in the in vivo section. Overall, this work suggests that STN HFS cannot be assumed to simply inhibit STN output, but it also provokes complex and contrasting synaptic changes within this nucleus.

Other Effects of STN HFS

Compared to electrophysiological experiments, very few studies on STN HFS using other techniques have been performed in vitro. In this context, the group of Moser have performed HFS on rat slices. They showed that HFS (130 Hz; 600 μA; 60 μs) reduces dopamine outflow in the striatum by activating a presynaptic GABAergic inhibitory control via $GABA_A$ receptor [94]. Moreover, this group showed that this control is probably due to an enhancement of extracellular striatal GABA, by an inhibitory effect of HFS on GABA uptake system rather than a stimulation of vesicular GABA release [95]. Their data

suggest that GABAergic transmission could be a preferential cellular support to the effects of HFS, as recently mentioned about the impact of the STN HFS on the output structures of the BG.

In parallel, to determine the molecular mechanism of HFS, the group of Berger has investigated the cellular effects of HFS and low-frequency electrical stimulation on prolactin secretion in GH3 cell lines (prolactinoma) as well as on catecholamine secretion of PC12 cells (pheochromocytoma). Their study demonstrated that HFS can produce an inhibitory effect on the cellular mechanisms responsible for the production and release of molecules participating in intercellular communication. However, in this model of isolated cultured cells where no network interactions are present, the observed effects are limited to the intrinsic properties of the cells [96].

These two kinds of biochemical studies report that HFS could act not only on cellular network but also on intrinsic molecular properties of the cell.

Main Contributions of In Vitro Approaches

The debate around the inhibitory vs. excitatory effect of STN HFS on the activity of this nucleus that has begun in the last decade can now, at least in part, be set up, since these effects are more complex than a decrease or an increase of STN neurons firing (see Table 21.1). What seems to emerge from in vitro electrophysiological studies is that HFS disrupts the peacemaker-like activity of STN neurons by acting on the intrinsic membrane properties of these cells. If we stick to stimulation frequencies relevant to clinical use, this will result in a temporary inhibition of STN firing after brief (seconds) stimulations and also, and most importantly, in a more complex phenomenon during longer periods of HFS (minutes to hours): a complete replacement of spontaneous STN activity by a stimulus-driven one. However, more complex mechanisms involving synaptic transmission within the STN cannot be excluded. These evidences support the hypothesis that STN HFS can disrupt the abnormal synchronized oscillatory activity of the subcortical–cortical loops in parkinsonian state, thus restoring a more physiological functioning of these structures and improving motor symptoms. On the other hand, it should be noted that all the electrophysiological studies were performed on very young or postnatal animals, where the structural and functional development of the brain synaptic network is still ongoing. It would be interesting to test whether in slices from adult rodents similar results could be obtained. Finally, the few data obtained in PD models showed no differences in STN HFS effect compared to controls. This is potentially interesting, since it could imply that HFS bypasses the basal ganglia dopaminergic system to exert its effects on neuronal activity in the STN. If one could reply that slices lack the connectivity of the whole brain, surprisingly similar findings were confirmed by in vivo reports (see below).

Table 21.1 Summary of the main known effects of experimental STN HFS (~130 Hz) on neuronal activity in the BG

Reference and model	Effect on STN	GPi	SNr	Str	Details of the effect
E, in vitro, rat [88]	–				After HFS, total or partial firing activity block
E, in vitro, rat [64]	–				During HFS, depolarization and spikes followed by total block
E, in vitro, rat [89]	–				During HFS, depolarization and spikes followed by total block
E, in vitro, PD rat [91, 92]	±				HFS-driven spike activity up to 130 Hz
H, ex vivo, PD rat [71, 97, 98]	–	–	–		Reduction of COI and GAD67 mRNA (previously increased by dopamine depletion)
E, ex vivo, PD rat [108]				–	Reduction of sEPSC frequency and NMDA receptor sensitivity
E, in vivo, PD rat [71]	–				Firing rate is generally inhibited
E, in vivo, PD monkey [74]	–				Firing rate is inhibited
E, in vivo, rat [105]	–		–		Firing rate is generally inhibited
E, in vivo, monkey [125]		±			≥136 Hz; from irregular to regular firing pattern
E, in vivo, PD rat [122]	–	±	±	±	Discontinuous HFS; spike activity is similarly inhibited and increased
I, ex vivo, rat [100]	+		+		Early gene induction

The column "Reference and model" reports, besides the reference, also the experimental approach (*E* = electrophysiology; *H* = in situ hybridization; *I* = immunohistochemistry), the specie, and whether or not the animal was rendered parkinsonian (PD). The column "Effect on" reports the resulting effect of STN HFS in terms of excitation (+) or inhibition (–) of the corresponding structure (STN = subthalamic nucleus; GP = globus pallidus; SN = substantia nigra; Str = striatum). The column titled "Detail of the effect" briefly resumes the effects of STN HFS.

High-Frequency Stimulation of the STN: In Vivo Approaches

Although in vitro studies provide the possibility of characterizing the synaptic and membrane response to STN HFS, in vivo approaches are necessary for investigating the widespread effects of DBS in the brain in terms of metabolism, neuronal activity, protein expression, etc. Also for in vivo approaches, however, there is a high variability in the experimental conditions utilized: DBS protocols are extremely heterogeneous, as well as the brain structures in which the consequences of STN HFS are examined. Moreover, in some cases DBS is delivered

in vivo but the effects are studied on ex vivo preparations (brain samples, slices, etc.). For these reasons, the available data on the effects of STN HFS in animal PD models have been separated into two main categories: ex vivo and in vivo; each contribution will be presented separately.

Effects Ex Vivo

In this section, we will present animal models of STN HFS in which DBS was performed in vivo and the effects assessed on in vitro preparations, i.e. ex vivo. The main difference compared to in vivo studies is that ex vivo provides a "static picture" of the consequences of DBS on a given structure and/or parameter, rather than the continuous monitoring of HFS effects that can be obtained by in vivo approaches. However, in the context of DBS, ex vivo has the advantage, compared to in vitro, that HFS is delivered in the intact brain of a living animal rather than on a preparation such as the slice that obviously does not include the whole brain connections. Moreover, ex vivo approaches give the possibility to easily perform investigations that would be technically impossible or extremely challenging if done in vivo.

Brain Metabolism

Actually, only one study concerned the effects of STN HFS on 2-deoxyglucose (2-DG) uptake in BG structures [97]. This experiment was done in MPTP-treated monkeys. The authors showed that dopamine lesion induced a decrease of 2-DG accumulation in the STN and an increase in the GPi, external segment of the GP (GPe) and ventrolateral thalamus, which were reversed by chronic STN HFS lasting for 10 days. Despite the significance of 2-DG uptake in term of excitatory or inhibitory influence and cellular elements involved not being clear, this study concluded that STN HFS could normalize the abnormal responses of GB structures to DA lesion. The reversal of STN response by HFS could reflect a hyperactivity of inhibitory GABAergic input in the STN which could counteract, as already suggested, the post-lesional STN reactivity. The increase of 2-DG in the GP after DA lesion may be due to the hyperactivity of excitatory input from the STN on these two structures, and the reversal of these enhancements under HFS could reflect the reduction of STN activity by the stimulation, as already shown by different studies [71, 98, 99].

Neuronal Activity and Plasticity in the STN

We first showed that STN HFS (130 Hz, 80 μs, 200 μA) applied for 2 h in normal and parkinsonian awake rats induced c-fos expression specifically in the stimulated STN [98]. A recent study in anaesthetized control rats [100] confirmed our results and further showed that STN HFS for 4 h (130 Hz or 80 Hz,

60 μs, 300 μA) also induced c-jun and krox-24 in stimulated STN neurons. Interestingly, the authors reported that this stimulation applied at 5 Hz did not induce the expression of this early gene in the STN. This immediate-early gene induction suggests that STN neurons are the preferential target of HFS but is unable to inform us about the nature of HFS influence on these neurons. To answer this question, we measured mRNA expression of cytochrome oxidase subunit 1 (COI), which is the terminal enzyme of the mitochondrial electron transport chain. COI mRNA expression indicates the level of neuronal metabolism [101, 102], and modifications in COI mRNA expression in different BG nuclei correspond to changes in firing rate and firing pattern [103]. Our studies and others reported that STN HFS (130 Hz; 80 μA for 2 h to 5 days in awake rats; or 400 μA for 45 min in anaesthetized rats) decreased or reversed the increase induced by DA lesion in the stimulated STN [71, 98, 99, 104]. The increase of metabolic activity in the STN after DA lesion is correlated with a change of firing pattern (from a regular pattern of discharge to a bursting activity). This decrease of COI mRNA expression in the STN under HFS could reflect a reduction of the firing rate by HFS as observed by electrophysiological studies [71, 105].

Neuronal Activity and Plasticity Outside the STN

Different studies reported that the increases of GAD67 mRNA expression (enzyme of GABA synthesis) and COI mRNA expression in the SNr, detected after dopamine lesion, are suppressed by STN HFS [71, 98, 99, 104]. We observed that 2 h continuous STN HFS could reverse the post-lesion responses in the SNr but not in the striatum and GPe [98]. These data could be correlated with the early gene induction in SNr neurons after STN HFS [100], as well as the decrease of electrophysiological activity of SNr neurons after STN HFS [71, 106]. Moreover, these results also suggest that the therapeutic effects of STN HFS are mediated by the normalization of SNr activity.

Considering the GPi (in primates) or entopeduncular nucleus (EP, in the rat), the other output structure of the BG, the effects of STN HFS differ from those observed in the SNr. Indeed, a partial reversal of the post-lesional GAD67 mRNA increase is observed in the EP of parkinsonian rats after STN HFS for 2 h and 3 days [98, 99]. Our preliminarily data showed that STN HFS applied for 6 days is not anymore able to counteract the dopamine depletion-induced increase of COI and GAD67 mRNA expression in the EP. This observation fits with recent experiments showing an increase of COI expression in GPi after 10 days STN HFS in MPTP-treated monkeys [97]. These data suggest that the short-term effect of HFS on EP neurons disappears with prolonged (days) stimulations. Conversely, STN HFS applied for 3–6 days, but not 2 h, antagonizes the dopamine depletion-induced increase of GAD67 mRNA in the GPe [98, 99]. Altogether, these results show an inverse time course of HSF effects in the two pallidal nuclei, suggesting a possible plasticity of BG network under stimulation.

Finally, we did not show any cellular effect of STN HSF in the striatum. Indeed, the increase of preproenkephalin mRNA and the decrease of preprodynorphin and preprotachykinin observed after dopamine lesion are not affected by the STN HFS [98, 99, 107]. This finding suggests that the striatum is not a preferential target of the STN HFS and its therapeutic effects are mediated by the normalization of activities of the BG structures downstream striatal network. However, we also reported that STN HFS could modify the spontaneous glutamatergic activity of striatal efferent neurons [108], suggesting that the modulation of striatal neuronal activity by HFS may not result in changes of neuropeptide expressions in these neurons.

Interestingly, a recent study has investigated the effects of STN HFS (130 Hz, 60 μs, 500 μA) applied for 3 h on a large screen of gene expression in 1 mm sagittal brain sections including BG structures by using DNA microarray [109]. It reported a decrease of calcium/calmodulin protein kinase type IIA and Homer1 suggesting a potential reduction of glutamate transmission in the BG network. This study also showed that IGF-2 and insulin-like growth factor-binding protein 2 (IGFBP2) are increased after HFS, which would underlie a possible reorganization of the neuronal BG circuitry.

Neuroprotective and Dopaminergic Effects of STN HFS

Several ex vivo studies have been done to evaluate the potential effect of STN HFS on the dopamine DA neuron survival, testing the hypothesis that silencing STN activity by HSF could be a method for neuroprotection in PD. Maesawa and collaborators [110] showed that when STN HFS (130 Hz, 60 μs, 80–100 μA) was applied continuously for 2 weeks, prior partial dopamine lesion induced by 6-OHDA intrastriatal injection partially prevented dopamine neuronal loss. However, this study does not provide a clinical relevance since HFS preceded dopamine lesion, whereas STN HFS in humans is performed when dopaminergic degeneration is ongoing. Alternatively, a recent report showed that bilateral STN HFS (130 Hz, 60 μs, 30 μA) applied for 1 h per day, starting a week after 6-OHDA injection and during a period of 3 months, increased the survival of midbrain dopaminergic neurons in the 6-OHDA rat model of PD [111]. Their stereologic analysis demonstrated that HFS had a protective effect on the number of dopamine neurons as well as on the total number of neurons in the SNc (by cresyl violet staining), suggesting a real neuronal sparing and not a change of tyrosine hydroxylase neuronal phenotype. Accordingly, a recent finding showed that continuous STN HFS (131 Hz, 52 μs, 50 μA), applied for 2 weeks and initiated 5 days after 6-OHDA lesion, preserves 30% of nigral neurons expressing tyrosine hydroxylase as compared to sham animal with electrode implantation in the STN [112]. Another study also indicated that STN HFS (130 Hz, 60 μs, 2–3 V) in MPTP monkeys offered 20–24% neuroprotection to dopaminergic cells [113]. Meissner and colleagues [114] reported that STN HFS increases striatal tyrosine hydroxylase activity without

affecting its gene expression. Moreover, STN HFS has been shown to enhance striatal tyrosine hydroxylase activity by increasing its phosphorylation [115]. Although clinical findings reported that STN HFS fails to improve dopamine outflow in PD patients [116, 117], most of the studies in animal models of partial dopamine lesion are in agreement with an activation of dopaminergic transmission by STN HFS, suggesting that HFS may act on the remaining dopamine neurons that are not anymore present in late PD stage, when the patient is undergoing HFS.

An important question concerns the interaction between STN HFS and L-DOPA treatment, since PD patients received dopaminergic treatments several years before being stimulated. L-DOPA treatment is known to induce disabling side-effects after several years, such as abnormal involuntary movement disorders (dyskinesia). In this context, we showed that STN HFS (130 Hz, 80 μs, 80 μA during 5 days) exacerbated the behavioural and cellular changes (neuropeptide mRNA expression) produced in the striatum by a dyskinesiogenic L-DOPA treatment, including the responses that are sustained after L-DOPA withdrawal [107]. This finding argues against a direct antidyskinetic effect of STN HFS and supports the recent clinical view that the relief of dyskinesia by this surgical treatment is due to the postoperative reduction of dopaminergic medication [24, 118].

Electrophysiological Effects of STN HFS

The first study reporting long-term effects of STN HFS on the electrophysiological activity of striatal neurons has been performed by our group [108]. Indeed, we examined the consequences of 5 days chronic DBS on the spontaneous glutamatergic activity of striatal projection neurons and on motor performance of parkinsonian rats (intranigral 6-OHDA injection). The animals were unilaterally stimulated by a bipolar platinum–iridium electrode (500 μm tips, 76 μm diameter, 500 μm distance between the two wires). DBS consisted of rectangular current pulses at 130 Hz, 80 μs width and 60 μA intensity. Such chronic stimulation was able to ameliorate the akinetic symptoms of parkinsonian rats, assessed by the cylinder test. Interestingly, this motor effect was paralleled by a strong reduction of glutamatergic activity of striatal projection neurons, recorded by patch-clamp techniques from brain acute slices. It is known that glutamatergic activity in the striatum recorded in vitro is increased by 6-OHDA lesion, and in vivo data seem to support such findings [108, 119, 120]. Thus, these changes of corticostriatal glutamate transmission seem to correlate with 6-OHDA-induced akinesia. It should be mentioned that glutamatergic hyperactivity and akinesia induced by 6-OHDA lesion are also reversed by STN lesion [121], suggesting that similar mechanisms might underlie the motor and synaptic effects of STN lesion and STN HFS.

Effects In Vivo

Electrophysiological Effects of STN HFS in the STN

The first report showing the electrophysiological effects of STN HFS in vivo on STN neurons was provided in naïve anaesthetized rat by Benazzouz et al. [105], showing that after 5 s of DBS (at 130 Hz, 500 μs, 10–1000 μA, concentric bipolar electrode) the activity of these cells was inhibited for 30–90 s. The same group lately investigated DBS effects in unilaterally 6-OHDA-lesioned rats [71]. Electrophysiological recordings were performed extracellularly by a glass microelectrode from animals anaesthetized with urethane. In parkinsonian animals, STN neurons showed no average changes in the mean firing rate compared to controls. However, the percentage of irregular firing STN neurons increased in the 6-OHDA-lesioned group (Fig. 21.5). DBS was delivered at 130 Hz (pulse width 60 μs and current intensity 400 μA) by a concentric bipolar electrode (200 μm tip diameter), similar to those utilized in humans. For these electrophysiological experiments, HFS lasted 10 s and recordings were performed during HFS. In control rats, DBS induces a significant inhibition of the firing rate in 72% of STN neurons, a complete suppression in 15%, an increase in 4%, and 9% is not affected by HFS. In 6-OHDA rats, DBS induces a significant inhibition of the firing rate in 61% of STN neurons, a complete suppression in 16%, an increase in 5%, and 18% is not affected by HFS. Interestingly, this group also utilized lower HFS frequencies (1, 10 and 50 Hz), finding that the response to DBS is frequency dependent: the higher the frequency, the higher the percentage of neurons showing changes in their spike activity. A similar inhibitory effect on the activity of STN neurons has been provided by Shi and colleagues [122] in behaviourally active rats with unilateral 6-OHDA lesion (this group also examined the effect of STN HFS in other structures of the BG, see below). In their study, DBS was delivered by 2 platinum–iridium microwires (50 μm diameter, 250–500 μm, 200 kΩ) of an array of 10, from which 8 were utilized for extracellular recordings in the STN. HFS was delivered at 130 Hz, 60 μs pulse width and 50–175 μA, in 3 s "on" and 2 s "off" stimulation cycles during 20 s treadmill walking phases, in which the locomotion impairments due to 6-OHDA lesion were improved. This behaviourally active STN HFS induces a decrease in the firing rate in 62% of the recorded STN neurons ipsilateral to the stimulating electrode, and in 26% of contralateral, while few (3.2 and 6.5%, respectively) show an increased discharge rate. This inhibition persists both during the 3 s "on" and 2 s "off" HFS cycle, thus during the whole 20 s stimulation. In parallel, the analysis of burst activity during the 2 s "off" cycles revealed that HFS significantly reduces burst rate in the STN. However, the use of on/off stimulation cycles prevented the observation of effects lasting more than 2–3 s, and it can be argued

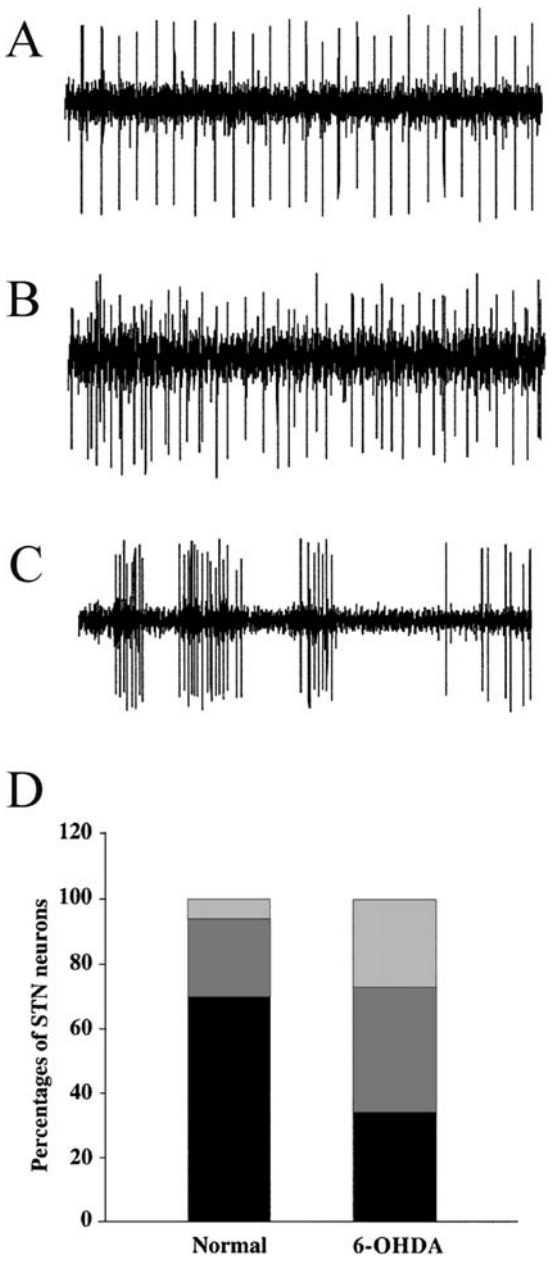

Fig. 21.5 In vivo single unit extracellular recordings of STN neurons. (**A**) Spontaneous and regular spike activity of a STN neuron from a control rat. (**B**) and (**C**) Spontaneous spike activity of two STN neurons from parkinsonian rats (6-OHDA lesion in the SNc): irregular (**B**) and burst (**C**) firing is observed in these animals. (**D**) The percentage of burst (*light grey*), irregular (*grey*) and regular (*black*) firing neurons is different between control and parkinsonian rats (modified from [71])

that such cyclic stimulation has no equivalent in other papers or in clinical application.

The effect of STN HFS on the activity of STN neurons has also been investigated in a primate PD model (two rhesus macaques treated by MPTP)

by Meissner and colleagues [74]. In parallel, the efficacy of this treatment was assessed by measuring contralateral rigidity on a modified UPDRS scale. The two monkeys were implanted with four glass-coated tungsten microelectrodes, of which three were used for electrophysiological recordings and one for DBS at 130 Hz, 100 μA and 60 μs pulse width. Each recording session included 6 min before, 6 min during and 9 min after stimulation. The mean spike frequency of STN neurons was not significantly affected by MPTP lesion, in contrast with previous data [72, 73]. However, MPTP treatment significantly increased the number of individual STN neurons with oscillatory activity. As observed in 6-OHDA-lesioned rats [71, 105] and in PD patients [123, 124], the firing rate of STN neurons was inhibited by STN HFS. One interesting report of Meissner's work [74], however, is that the spikes triggered by STN HFS are not time locked to the electrical stimulus as observed in vitro. In particular, in most of the recorded cells the first spike after the artefact occurs about 3 ms after the artefact, which is almost half of the whole inter-stimuli interval: this means that these cells are silenced for 3 ms or more after each stimulus. The firing rate returns to baseline values only ~7 ms after the stimulus, thus just about 1 ms before the subsequent stimulus. Another effect of STN HFS is to decrease the number of individual STN neuron oscillations, but without affecting the oscillation frequency (τ and α). Concerning the correlated activity between pairs of STN neurons, this is not modified by MPTP lesion but it is reduced by DBS. This reduction of the abnormal MPTP-induced oscillatory activity by STN HFS may thus result in a subsequent decrease of abnormal oscillations of the cortico-BG-cortico loop, presumed to play a role in the motor symptoms of PD.

Electrophysiological Effects of STN HFS Outside the STN

In the above-mentioned paper by Benazzouz et al. [105], the authors also examined the effects of STN HFS in the SNr of both control and 6-OHDA-lesioned rats and in the ventrolateral nucleus of the thalamus of controls. SNr neurons respond to STN HFS (5 s, 130 Hz, 300 μA) with a significant decrease in firing rate, lasting 50–160 s. This effect is similar in both parkinsonian and naïve rats. Conversely, neurons of the ventrolateral nucleus of the thalamus respond with a significant increase in their activity, which also lasts for a while (25–150 s) after DBS is turned off.

The MPTP primate model of PD is probably the most suited to study the widespread effects of STN HFS with an electrophysiological approach and, accordingly, one of the first experimental works has been performed in the primate by Hashimoto and collaborators [125]. Two rhesus macaques were implanted unilaterally by a scaled-down version of the electrode used in humans, consisting of four metal contacts separated by 0.5 mm, each with 760 μm diameter and 0.5 mm thickness, with a resistance of 100–150 MΩ.

Stimulation was provided with parameters adjusted in each monkey, and electrophysiological extracellular recordings were performed from the GPe and the GPi by platinum–iridium electrodes. Interestingly, Hashimoto and colleagues first examined the effects of DBS on parkinsonian motor signs, not only in order to assess the efficacy of this treatment to ameliorate these symptoms but also to find the effective stimulation parameters. In both monkeys, 136 Hz DBS could ameliorate contralateral spontaneous limb movement and rigidity, without inducing dyskinesia. Several voltages were tested, and only those at the higher range (2.4–3.5 V) were effective, and pulse duration was set to 210 μs for one monkey and 90 μs for the other. Low-frequency (2 Hz) DBS produced a short-term response in GP neurons consisting of a wave of five consecutive inhibition and excitation components. At 136 Hz and higher, the second stimulus of a given couple of stimuli coincided with the latter components, giving rise to a wave parameter in four phases (inhibition–excitation–inhibition– excitation). The overall result was that STN HFS changed the spontaneous irregular firing pattern of both GPe and GPi into a high-frequency regular pattern (Fig. 21.6). Stimulation usually lasted 30 s, during which a run-down of the average frequency was observed (Fig. 21.6), but longer DBS periods (>5 min) revealed that the increase in the mean discharge rate lasted during the whole stimulation.

A wide study on the effects of STN HFS in the BG ganglia of parkinsonian rats has been provided by Shi et al. [122] who recorded extracellularly from ipsi- and contralateral striatum, GP and SNr (see above for the stimulation parameters and protocol). In average, ipsilateral striatal neurons respond to DBS, showing an equal amount of neurons increasing or decreasing their firing rate during the 3 s "on" period, while some have a rebound of excitation just at the beginning of the 2 s "off" phase; contralateral neurons, conversely, are more excited in both periods. Overall, about 30% ipsi- and 15% contralateral striatal cells respond to DBS. GP neurons, both ipsi- and contralateral, show similar rates of percent inhibition and excitation, the latter being of a prevalent rebound type during the "off" period; overall, less than 20% of GP neurons responded to DBS. In the SNr, around 40% of neurons are affected by STN HFS, with an equal amount of excitatory and inhibitory effect during the "on" phase. This finding is somehow in contrast with a previous report by Maurice et al. [106], obtained in control rats under urethane anaesthesia, where STN HFS resulted in the inhibition of ~65% of SNr neurons, while ~22% were excited. The stimulation consisted of pulses of 60 μs of 1–15 V (corresponding to 20–300 μA intensity) delivered at 50–200 Hz for 30 s. Interestingly, this inhibitory vs. excitatory effect is correlated with, respectively, stimulation intensity below and above 4 V. Another interesting finding is that some ~13% of SNr neurons are antidromically activated by STN HFS at all stimulation intensities, and the antidromic spike follows with high fidelity the stimulation at 130 Hz, up to 600 Hz.

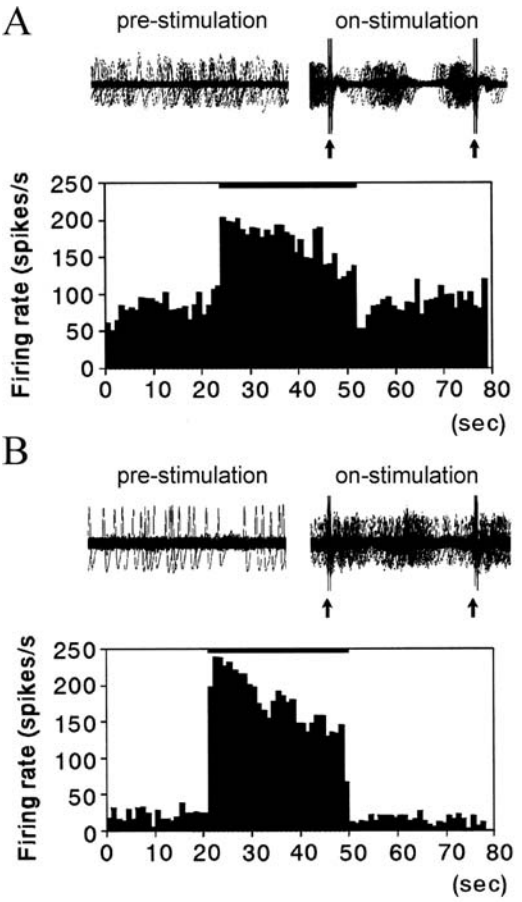

Fig. 21.6 Neuronal responses of GP to HFS STN in the rhesus monkey in vivo. Traces are 10 ms sweeps of neuronal activity of a GPi (**A**) and a GPe (**B**) neuron, before and during DBS. Arrows represent the residual stimulation artefacts. Note how neuronal activity is increased and, more notably, regularized, by 136 Hz STN HFS. The plots show the mean firing rate (calculated every 1 s) before, during (*black bar*) and after STN HFS: firing activity increases immediately when DBS is turned on and then slightly decreases during stimulation (modified from [125])

The stimulation of STN can activate, besides STN itself and its "classical" target structures, also the PPN, which has been recently proposed as a possible alternative target for DBS in PD. PPN is reached by a contingent of STN output fibres which, although less numerous than those projecting to the SNr and GPi, may have a relevant role in mediating the positive motor effects of STN HFS. For this reason, Florio et al. [126] have investigated the effect of STN HFS in the PPN of parkinsonian rats anaesthetized by chloral hydrate. Coaxial stainless steel electrodes (250 μm diameter, tip-barrel distance 200 μm) were used for DBS, whose parameters were 130 Hz, pulse width 60 μs, intensity 10–1000 μA, duration 1–5 s, with extracellular recordings being performed 10 s before HFS, during HFS and 20 s after HFS. These authors classified PPN neurons in three groups according to their firing properties in control rats: type 1 (39.7%), showing a rather regular firing pattern; type 2 (44.9%), with an irregular activity; type 3 (15.4%), with an oscillatory activity consisting of alternate

periods of bursts and silences. Dopamine lesion of the SNc by 6-OHDA reduces the number of type 1 neurons, decreases the mean activity of type 2 and has no effect on type 3. STN HFS in control rats affects 39.4% of PPN neurons, of which 84.6% are inhibited and 15.4% activated just after the end of the 1–5 s stimulation period. Such inhibition/activation lasts for about 3.6/14.8 s, respectively, and it is not dependent on the duration of the stimulation. On the other hand, roughly one-third of the inhibited neurons are activated during HFS. The percentage of PPN neurons responding to DBS (35.4%) is not affected by 6-OHDA lesion, and again the main result of STN HFS is the inhibition of neuronal activity in 90.9% of the recorded cells, similar to control rats.

Another structure that has been studied in the context of the widespread brain effects of STN HFS is the dorsal raphe nucleus (DRN), a midbrain structure providing extensive 5-hydroxytryptamine (5-HT) innervation to the limbic forebrain. The rationale for such study is that STN HFS may inhibit the activity of DRN neurons, resulting in a decreased release of 5-HT into the limbic forebrain, and thus triggering the psychiatric disorders mentioned before. In this context, Temel et al. [127] have performed extracellular recordings from the DRN of anaesthetized rats undergoing bilateral DBS of the STN, both in control and parkinsonian state (6-OHDA lesion). Stimulation was carried on for 2–3 min, and recordings continued for 2–3 min after HFS. Gold-plated coaxial electrodes, with an inner wire of platinum–iridium, were used, with a 250 μm shaft diameter, a tip of 50 μm diameter and 75 μm length, and an inter-pole distance of 50 μm. Stimulation parameters were variable in terms of frequency (10, 50, 100 and 130 Hz) and intensity (3, 30, 100 and 150 μA), while pulse width was constantly kept at 60 μs. The neurons recorded from DRN had the characteristic slow and regular (0.7 ± 0.1 Hz) firing pattern of 5-HT cells. Bilateral STN HFS inhibited by 45.1% the firing activity of more than 90% of DRN neurons. This effect had a rapid onset during DBS and quickly reversed to baseline after turning off the stimulation. Interestingly, such inhibition occurred at stimulation parameters relevant to clinical application, i.e. at >100 Hz and 30 μA, but not at <50 Hz. In parkinsonian rats, where the basal firing of 5-HT neurons is increased, STN HFS also inhibited 5-HT cell firing by 52.4%, similar to that observed in control animals. Interestingly, the intra-STN injection of muscimol, a $GABA_A$ receptor agonist, mimics the inhibitory effect of DBS on 5-HT neurons, suggesting that STN HFS reduces the output of this nucleus. These findings provide further support to a functional link between STN and 5-HT neurons of the DRN.

A possible action of STN HFS could occur through the antidromic activation of the axon collaterals of cortical pyramidal neurons projecting to the STN, thus stimulating local cortical circuits. This hypothesis has been explored by Li et al. [128] in the rat, both in control and in parkinsonian state. This group utilized two kinds of stimulating electrodes. One obtained commercially (250 μm tip diameter, 1 mm tip distance) and the other custom-made: the latter was a bipolar stainless steel electrode, with 81 μm diameter wires that were sharpened at the tip, with a resistance of 20–40 kΩ. Stimulation was provided in

biphasic (positive, negative) square waves at 40–160 Hz, amplitude of 80–260 μA, and applied for 0.8–2 s. Intracellular recordings show that a small group (15.6%) of layer V/VI cortical neurons respond to STN HFS with an antidromic spike, whose frequency reflects that of DBS with a latency of ∼2 ms (Fig. 21.7A, B). Another group of neurons, while not being directly activated by STN HFS, is modulated in terms of membrane potential: the overall effect is a significant reduction of the down state time during DBS, also with a marked reduction of depolarization/hyperpolarization potentials during, respectively, the up and the down state. A possible mechanism could be that antidromically stimulated neurons activate local excitatory and inhibitory cortical networks, thus dampening the slow-wave up–down activity typical of the anaesthetized

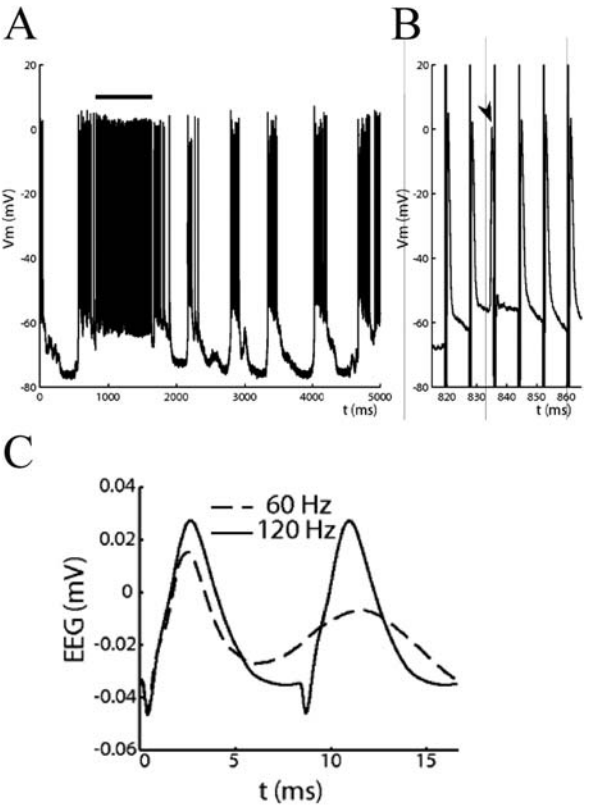

Fig. 21.7 Antidromic activation of motor cortex by STN HFS. (**A**) Example of an in vivo intracellular recording from a rat motor cortex neuron, depicting the antidromic activation by STN HFS. (**B**) Close up of A (see time scale) showing how spikes shortly follow DBS artefacts (2.0 ± 0.5 ms latency). The arrowhead shows a spontaneous spike that inhibits (by collision) the antidromic one expected after the stimulus. (**C**) Comparison between averaged EEG responses to DBS at different frequencies (2.6 ± 0.5 ms latency). Note the two separate response peaks at 60 Hz following stimulus *a*, and the superimposition of the primary peak of stimulus *b'* with the secondary peak of stimulus *a'*, leading to a resonant response (modified from [128])

rodent. The analysis of current source density of local field potentials, recorded at different depths of the motor cortex, reveals that excitation due to DBS reaches the deep cortical layers with a delay of ~2.5 ms, then spreads to the more superficial layers within the following ~5 ms. Electroencephalographic (EEG) recordings show that a single stimulating pulse delivered to the STN triggers two positive peak responses (Fig. 21.7C), separated by a gap of ~8.5 ms (the first with ~2.8 ms latency from the stimulation). Thus, at DBS frequency around 100–120 Hz, in which the inter-stimulus interval is around 8.5 ms, each stimulus occurs exactly at the time in which the two peaks originate, resulting in a resonant superimposition of the primary peak of a given stimulus with the secondary peak of the preceding stimulus (Fig. 21.7C). Li and colleagues also showed that STN HFS is able to dampen the slow-wave oscillations recorded by EEG and local field potentials from rats under deep anaesthesia [95]. The δ, τ, α and low β bands are actually depressed during stimulation on periods, and this effect is consistent with the above-described antidromic activation of cortical areas. This finding further supports the idea that cerebral cortex is involved in the mechanism of action of STN HFS delivered at 100–120 Hz, as proposed by several studies in patients showing that stimulating the STN produces evoked potentials in the frontal cortex [129, 130], and that direct stimulation of the motor cortex alleviates parkinsonian symptoms in both primates and humans [131, 132]. Both the enhanced EEG responses and firing rate observed by Li and colleagues during short periods of DBS show a rapid rundown during longer stimulation periods (100 s), reaching a steady state at roughly 30% of the initial potentiation, suggesting that such effects of DBS can operate for longer periods of time.

Biochemical Effects of STN HFS

Actually, as far as we know, no in vivo investigation is available about the biochemical state of STN during HFS in parkinsonian animals. The size of the STN and the implantation of the electrode prevented introducing microdialysis or voltametric probes in the stimulated nucleus.

In the SNr, microdialysis approaches (130 Hz, 60 μs, 500 μA during 3 h) showed that STN HFS induced an increase of extracellular GABA contents in hemiparkinsonian anaesthetized rats but doubled the level of GABA [133]. The enhancement of GABAergic inhibitory neurotransmitter levels could underlie the normalization by HFS of the nigral hyperactivity induced by dopamine lesion [106]. Windels and colleagues [133] suggested that this GABAergic increase may result partly from the stimulation of pallidonigral fibers, revealing a potential role for pallidal GABA in the inhibition of BG output structures during HFS STN.

In the striatum, STN HFS is reported to increase the dopamine efflux in rats with partial dopamine lesion [114, 134, 135]. Moreover, STN HFS, but not for example EP HFS, is reported to increase striatal dopamine efflux in rats with partial dopamine lesion [114, 134–137]. STN HFS (130 Hz, 60 μs, 300 μA in constant current mode for 20 min) also significantly and reversibly increased

extracellular levels of DOPAC and HVA in the ventral striatum of the rat with 6-OHDA lesion in the SNc [138], suggesting that STN HFS could promote dopamine transmission via VTA-accumbal pathway. Taken together, these results suggest that an enhanced dopamine release within the BG may be an important mechanism whereby STN HFS improves motor symptoms of early stage of PD. Thus, whether STN HFS improves Parkinson's disease symptoms at late stage via the release of dopamine remains a matter of debate. It is interesting to note that STN HFS prolongs the increase in striatal dopamine induced by acute L-DOPA treatment in dopaminergic denervated rats [139]. This synergic effect fits with our experimental evidences showing a potentiation of striatal cellular responses to L-DOPA by STN HFS [107].

Besides striatal dopamine transmission, it has been reported by an intracerebral microdialysis study that STN HFS induces an increase of extracellular glutamate and GABA contents in the striatum that are not DA dependent [140], suggesting that STN HFS effects are not only restricted to the direct STN targets.

Main Contributions of In Vivo Approaches

Delivering STN HFS on living animals has revealed itself technically challenging and opened several new questions. For example, the application of this technique to a small animal such as the rat imposed miniaturizing the electrodes and finding materials that were suitable for such application. The result was that, especially for the rat, several studies were performed utilizing bipolar electrodes, rather than monopolar ones such as those for clinical use. Concerning the stimulation parameters, in vivo studies have provided a major contribution for defining the range of current intensity that is experimentally effective, clinically relevant and, in particular, not inducing motor troubles itself. In fact, it is worth mentioning that HFS delivered intensities of 100–200 μA (by both mono- and bipolar electrodes) that can trigger a dyskinetic-like motor behaviour. This HFS-induced dyskinesia can mimic an anti-akinetic effect in parkinsonian animals and eventually can interfere with dyskinesia experimentally induced by L-DOPA [71, 98, 99, 107, 141]. Thus, it is of extreme importance to keep appropriate stimulating parameters not only in terms of frequency (around 130 Hz) but also in terms of intensity. The current density is also an important parameter when we want to compare to human studies. Current density is defined as the intensity of the current vs. the surface of the electrode. Indeed, the active surface of the electrodes used in animals is smaller than those used in human, thus it is crucial not to apply an intensity close to human studies in order to prevent any tissue damage. However, most of the ex vivo and in vivo studies have been done in anaesthetized animals and for a short duration of stimulation: unfortunately, these experimental conditions do not allow knowing if the current intensity can induce behavioural side-effects (dyskinesias, see above) and/or electrolytic lesions.

What can be concluded by summarizing in/ex vivo electrophysiological studies is that, in general, STN HFS inhibits the activity of the STN itself. The effect on the other brain structures, while generally also inhibitory, seems more complex than a simple inhibition (see also Table 21.1). Concerning the cortex, it can be antidromically activated by HFS, resulting in changes in its waveform activity patterns that could possibly affect both pyramidal and extrapyramidal pathways. Other brain structures that more anatomically correlated to the STN, such as the GP and the SN, seem to be generally inhibited in terms of spike activity and the striatum also in terms of synaptic glutamatergic activity. Another interesting finding of electrophysiological in vivo and ex vivo studies is that they confirm what has been reported by the few electrophysiological in vitro works performed in PD models, i.e. STN HFS has the same effect in parkinsonian and control animals. This is true for the direct effect in STN itself, as well as all the other brain structures examined. Again, as mentioned before, this could imply that STN HFS can bypass BG dopaminergic systems for exerting its effects, at least at cellular level. The data issued from biochemical studies confirm that STN HFS inhibits the metabolic activity of the STN. However, the cellular mechanism of this effect is still unknown. In the BG structures, where some effects of STN HFS could be explained by a reduction of excitatory STN influence, biochemical and electrophysiological data suggest that it is more complex, i.e. implying of pallidal GABAergic transmission in the effect of HFS in the SNr and differential effects of HFS in the three preferential targets (GPe, EP and SNr) of the STN. We now suppose that STN HFS impact could induce a real reorganization of the BG network involving neuroplasticity phenomena such as structural and functional changes including fibre sprouting and connectivity rearrangements.

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Chapter 22
Surgical Strategies for Parkinson's Disease Based on Animal Model Data: *GPi and STN Inactivation on Various Aspects of Behavior (Motor, Cognitive and Motivational Processes)*

Christelle Baunez

Introduction

In the previous chapter, Gubellini and Salin have introduced extensively why the subthalamic nucleus has been a selected target for the treatment of parkinsonism and what are the other target structures.

After a long period during which pharmacological treatments were the only option for the treatment of Parkinson's disease (PD), a short episode of graft implantations, the most recent treatment relies on surgical therapy. The choice of the target remains however variable, depending on the specific deficits of the patient. This chapter will focus mainly on two major targets and will omit the ventral intermediate nucleus (VIM) of the thalamus that was the initial target for the treatment of tremor in PD patients and the most recent one – the pedunculopontine nucleus – currently chosen to treat the freezing.

As a reminder, Fig. 22.1A represents a schematic of the basal ganglia organization that was used as a reference for many years, leading to the suggestion that STN could be an interesting target for parkinsonism, since it becomes hyperactive in case of DA depletion [1]. Figure 22.1B shows the basal ganglia organization as we look at it now, placing the striatum and the STN as the two major input stations of the basal ganglia [2]. It is interesting to note, however, that although this revised version of the diagram is more accurate, we still rely on the former one to explain most of the current findings in the literature.

This chapter will list for each target the behavioral effects of either lesions or DBS, when available in monkeys and rats. Motor, cognitive and motivational behavioral measures will be reported when available.

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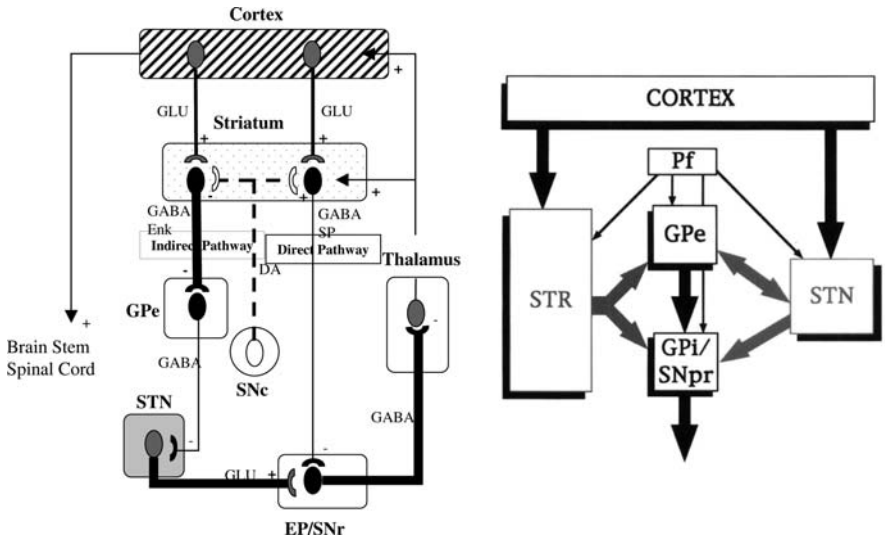

Fig. 22.1 (**A**) Schematic of the basal ganglia organization after a DA depletion as proposed by DeLong [1]. This diagram clearly indicates an hyperactivity of the STN, suggesting therefore that normalization of STN activity could be a beneficial treatment for parkinsonism. (**B**) Schematic organization of the basal ganglia proposed by Levy et al. [2] illustrating the hyperdirect pathway and therefore giving to STN a status of "major input to the basal ganglia"
STR: striatum, STN: subthalamic nucleus, GPe: external segment of the globus pallidus, EP: entopeduncular nucleus (= GPi: internal segment of the globus pallidus), Pf: parafascicular nucleus of the thalamus, SN(p)r: substantia nigra pars reticulata, GLU: glutamate, Enk: enkephaline, SP: substance P, SNc: substantia nigra pars compacta.

The Internal Globus Pallidus (GPi)

The internal segment of the globus pallidus (GPi) (or entopeduncular nucleus (EP) in the rodent) belongs to the "output structures" of the basal ganglia and is often associated indistinctively with the substantia nigra pars reticulata. It is a GABAergic structure innervating mainly the motor thalamic nuclei and receiving its two major inputs from the striatum (the so-called direct pathway) and the STN.

Motor Behavior

Lesion and Pharmacological Data in the Monkey

One of the first evidence showing that the GPi could represent an interesting target for the treatment of PD was provided by pharmacological experiments showing that blocking the glutamatergic transmission within the GPi alleviated

motor deficits in monkeys rendered parkinsonian with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [3–4]. Similar effect was observed in the unilateral model of MPTP parkinsonian monkey, in which a unilateral injection of MK801, an NMDA antagonist, into the GPi induced a contralateral circling similar to that induced by DA agonists [2].

Mimicking an inactivation is often performed by means of infusions of the GABA agonist muscimol into the given cerebral structure. In 1999, Wenger et al. [5] showed that a focal inactivation of the GPi with muscimol infusions impaired grasping and reaching, affecting velocity. The results supported the hypothesis that inhibition of the GPi disrupts the ability to inhibit competing motor mechanisms to prevent them from interfering with desired voluntary movement. In a later study where they tested the effects of muscimol infused into various selective areas of the GPi, Baron et al. [6] showed that akinesia and bradykinesia induced by MPTP could be alleviated when muscimol was infused into the centromedial part of the sensorimotor GPi [6]. The same study showed that inactivation of areas outside of the motor territories did not improve parkinsonism but induced circling and behavioral abnormalities.

Lesions of the GPi were rarely tested on behavior in monkeys. One of these studies used kainic acid lesions and revealed motor deficits in reaction time performance [7], while a study using kynurenic acid showed dyskinesia [8].

Although there are numerous clinical studies dedicated to the beneficial effect of pallidotomy on L-DOPA-induced dyskinesia in parkinsonian patients, there is little evidence in the monkey literature if we omit the indirect evidences from electrophysiological and immunohistochemical studies. Indeed, in the marmoset rendered parkinsonian with MPTP, it was shown that a unilateral electrolytic lesion of the GPi could reduce the L-DOPA-induced dyskinesias [9].

GPi HFS Data in the Monkey

High-frequency stimulation (HFS, also called deep brain stimulation or DBS) has been widely applied into the GPi of PD patients, but surprisingly, there are not many studies supporting this therapeutical approach. One pioneer study has shown that unilateral HFS of the GPi could improve obvious observable parkinsonian symptoms such as muscular rigidity and akinesia in unilateral MPTP monkeys [10]. Although it has been shown that GPi DBS applied in PD patients was efficient for the treatment of L-DOPA-induced dyskinesia (for review [11–12]), there is no published study to date showing this effect in monkeys.

Lesion Data and Pharmacological Manipulations in the Rat

In the reserpinized model, injection of glutamatergic antagonists into the EP restores locomotor activity [4]. In the same model, or in alpha-MPT model, NMDA antagonists injected into the EP also alleviate muscular rigidity [13]. In unilateral DA-depleted rats, lesioning the EP decreased the rotations induced by amphetamine or L-DOPA [14–15]. In another study, it was however shown

that an EP lesion was unable to correct the circling behavior induced by L-DOPA in unilateral DA-depleted rats [16]. It was also shown that excitotoxic lesions of the EP alleviate the cataleptic state induced by haloperidol [17].

In the intact rat, first we have shown that bilateral infusions of an NMDA antagonist, APV, into the EP can induce an akinetic-like deficit associated with a premature-responding deficit in a simple reaction time (SRT) task [18]. In order to measure the effects of intra-EP bilateral infusions of APV in a rat model of early parkinsonism, we have used a task allowing a subtle measure of simple reaction time task (see Fig. 22.2). In this task, the rats are trained to press

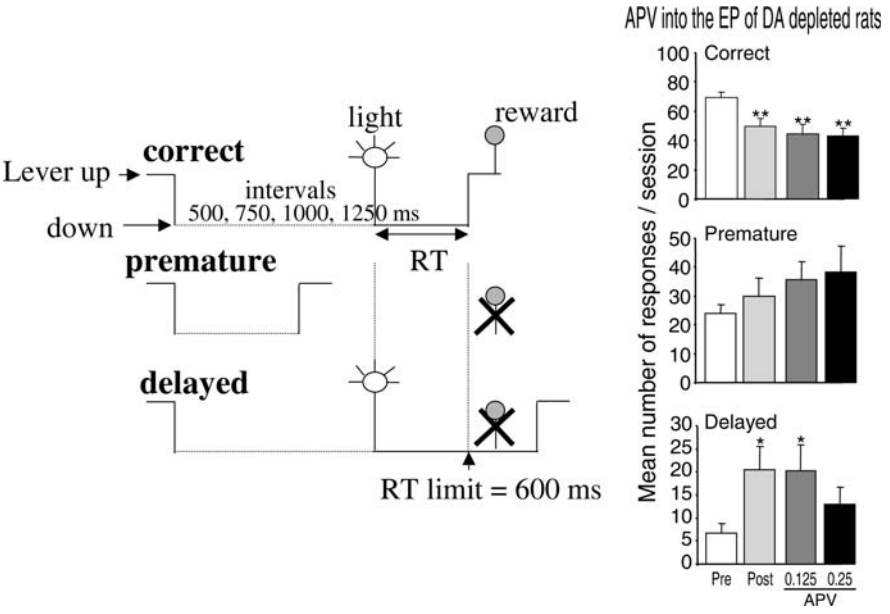

Fig. 22.2 **(A)** The simple reaction time task used in the rat. The rats are trained to press a lever down and sustain their paw on it until the occurrence of a visual stimulus (a light) that may happen at either 500, 750, 1000 or 1250 ms. At the occurrence of the light, the rats have to withdraw their paw from the lever as quickly as possible (i.e., reaction time, which has to be below 600 ms) to get a food pellet as a reward. Three types of responses are possible: (1) correct, (2) premature responses when the rat withdraws its paw from the lever before the occurrence of the light and (3) delayed responses when the rat withdraws its paw from the lever after the occurrence of the light, but with a reaction time exceeding 600 ms. **(B)** Effects of a bilateral infusion of NMDA antagonist into the EP of rats rendered parkinsonian by a bilateral infusion of 6-OHDA into the dorsal striatum. The performance is illustrated in terms of number of correct responses/100-trial session before the surgery (Pre), after the 6-OHDA lesion with NaCl infusion into the EP (Post), after APV infusion at the dose of 0.125 and 0.25 μg/0.5 μl infusion. The DA depletion induces an akinetic-like deficit characterized by an increased number of delayed responses and decreased number of correct responses. In these akinetic animals, the infusion of APV into the EP reduces the number of delayed responses, but the performance in terms of correct responses remains seriously impaired because of increased number of premature responses (Baunez and Amalric, data unpublished) *, **: significatively different from pre-operative performance; $p < 0.05$ and 0.01, respectively.

a lever down and sustain their paw on the lever until the occurrence of a light, at which they have to release the lever quickly to obtain a food pellet. The reaction time is the time taken to withdraw the paw from the lever after the onset of the light. Parkinsonian patients suffering from akinesia are known to exhibit increased RT in these tasks. After a bilateral infusion of 6-OHDA into the dorsal striatum, the rats' performance is impaired in terms of correct responses, mainly because of increased RT, resulting in an increased number of delayed responses (non-rewarded responses for which the RT exceeded 600 ms). As shown in Fig. 22.2, in this model of rat parkinsonism, we have shown that the same bilateral infusion of APV into the EP alleviates akinetic-like behavior in the SRT task in the rat by reducing the number of delayed responses (see Fig. 22.2; Baunez and Amalric, unpublished).

EP HFS Data in the Rat

Probably because of its small size in the rat, the EP has been rarely specifically targeted for HFS electrodes implantation. No behavioral study to date has been published regarding EP HFS effects. Only one microdialysis study has reported that EP HFS increases DA levels in the striatum when DA drugs are administered [19].

Cognitive Behavior: What Is Available in Animal Models?

Unfortunately, no study testing the effects of GPi/EP manipulation on cognitive functions has been published to date either in monkeys or in rats. Given the clinical reports after pallidotomy or GPi DBS, it would be very interesting to investigate further attentional functions as well as executive functions.

This first part dedicated to the GPi revealed that a large body of evidence supported the possible beneficial effect of pallidotomy or GPi DBS for the treatment of motor deficits in parkinsonism. However, this review of the literature reveals as well a serious lack in investigations of the non-motor functions. Although clinical application of GPi DBS in the treatment of PD does not seem to induce cognitive side effects, there are reports of mood changes and gain in weight that might be related to the direct consequence of GPi manipulation. It would therefore be useful to investigate further these observations in animal models.

The STN

The subthalamic nucleus (STN) belongs to the so-called indirect pathway of the basal ganglia and also to the "hyperdirect pathway". It is a glutamatergic structure innervating mainly the output structures of the basal ganglia (EP and SNr), but also the GPe and VP, the pedunculopontine nucleus, the striatum and

accumbens and the dopaminergic nuclei (ventral tegmental area (VTA) and substantia nigra pars compacta (SNc)). Its major inputs originate in the various cortical areas (i.e., hyperdirect pathway), the GPe and VP, the striatum (the "indirect pathway"), the parafascicular nucleus of the thalamus, the pedunculopontine nucleus, the dorsal raphe and the VTA and SNc (for a review, see [20]).

Motor Behavior

Lesion Data in Monkeys

If we consider the effects of STN lesion in an intact monkey, it was first reported that it induced a characteristic hyperkinetic syndrome called "ballism" or "hemiballism" [21]. The first paper showing anti-parkinsonian effects of STN lesions in a MPTP monkey was published by Bergman et al. [22] in 1990. It was based on serious motor impairment induced by MPTP that could be alleviated by STN lesions. The study was performed by means of general observation of gross motor behavior, with no measure of controlled operant responses. This pioneer study was further confirmed by the study by Aziz et al. [23]. In line with these reports, it was also shown that subthalamotomy performed in MPTP monkeys had a beneficial effect on certain motor deficits, but could also be detrimental by inducing hyperkinetic movements and hemiballism [24–26].

In the hemi-parkinsonian marmoset, it was also shown that unilateral subthalamic lesions reversed the bias in head position and decreased latencies to initiate reaching on the contralateral side in the staircase grasping task. However, slight deficits in skilled movements persisted [27]. Akinesia and bradykinesia were strongly ameliorated by discrete inactivation with muscimol of the lateral part of the sensorimotor territory in STN [6].

STN HFS Data in Monkeys

Benazzouz and colleagues [28] were the first to show that unilateral STN HFS applied in monkeys rendered hemi-parkinsonian with MPTP alleviated the muscular rigidity observed in the contralateral forelimb. This pioneer work was actually at the origin of the idea to apply STN HFS in PD patients.

In the intact monkey, it was also shown that STN HFS could induce hyperkinetic movements similar to the hemiballism observed after STN lesions [29]. In contrast to what was described after STN lesions, STN HFS does not seem to induce hyperkinetic movements when applied to MPTP monkeys and when compared to L-DOPA effects [30].

Lesion and Pharmacological Data in Rats

In intact rats, unilateral lesions of the STN only produce transient hyperkinetic movements of the contralateral paw; this behavior has been quantified by

measuring spontaneous circling behavior [31]. When the lesion is bilateral, this behavioral effect has been rarely described. Only a trend to hyperlocomotion has been reported, as well as premature responses in reaction time procedures [32].

In rat models of parkinsonism, it was first shown that lesions of the STN alleviated the cataleptic state induced by a high dose of haloperidol [17]. When performed unilaterally, STN lesions can reduce circling behavior induced by either a D2 agonist or apomorphine in hemi-parkinsonian rats [33–35]. These were some of the first rodent studies showing that STN lesions had a beneficial effect in alleviating gross motor deficits induced by a dopaminergic deficit. In line with these beneficial effects of STN lesions on these types of motor behavior, it was also shown that unilateral STN lesions could alleviate a postural asymmetry induced by unilateral DA depletion [36].

In order to measure the effects of bilateral STN lesions in a rat model of early parkinsonism, we have tested the effects of these lesions in parkinsonian rats performing the simple RT task described above (Fig. 22.2). As shown in Fig. 22.3, bilateral lesions of the DA terminals in the dorsal striatum increased

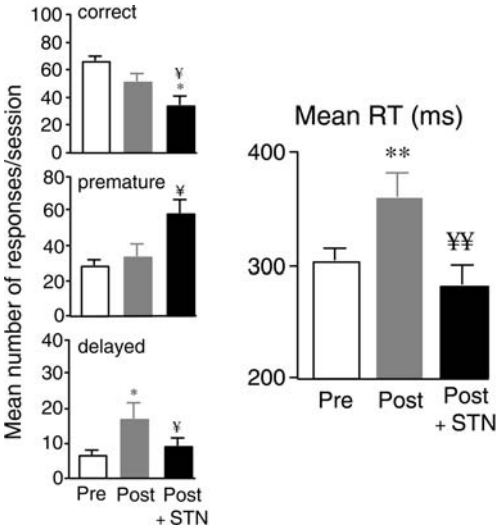

Fig. 22.3 Effects of STN lesions in a rat model of parkinsonism on the performance in the SRT task [32]. The performance is illustrated in terms of number of correct responses/100-trial session before surgery (Pre), after 6-OHDA lesion (Post) and after STN lesion consecutive to 6-OHDA lesion (Post + STN). As illustrated in Fig. 22.2, the dopaminergic depletion of the dorsal striatum induced an akinetic-like deficit characterized by an increased number of delayed responses (responses with an RT above 600 ms) and an overall increased RT for correct responses. Performing a bilateral lesion of the STN in these animals alleviated these two major deficits, but affected further the performance in terms of correct responses because of a dramatic premature-responding deficit

*, **: significatively different from pre-operative performance; ¥, ¥¥: significatively different from post-operative performance (6-OHDA lesion effect); $p < 0.05$ and 0.01, respectively.

the number of delayed responses, as well as the mean RT for correct responses, characterizing an akinetic-like pattern of performance. Consecutive bilateral lesions of the STN alleviate this akinetic-like deficit, but maintain a poor level of performance in the task due to the appearance of a premature-responding deficit [32]. Although this study confirmed the beneficial effect of STN inactivation on motor disabilities in PD, it also revealed for the first time possible side effects that might be related to the involvement of STN in non-motor behavior. These results were confirmed by a similar study carried out with unilateral lesions [37].

In another study, it was also confirmed that STN lesions do alleviate some of the deficits induced by a DA depletion, but induce side effects or are unable to correct some deficits such as a paw-reaching deficit assessed in a staircase grasping task [38].

STN HFS Data in Rats

The first study published on STN DBS in freely moving rats used unilateral STN DBS as well as unilateral DA depletion in the substantia nigra. The study assessed basic motor tasks such as haloperidol-induced catalepsy, apomorphine-induced circling behavior as well as a choice reaction time task [39]. The parameters were set at 130 Hz, 60–70 μs pulse width and intensity just below the threshold of hyperkinetic movements of the contralateral paw. In this study, we have shown that both cataleptic states induced by haloperidol and circling behavior induced by apomorphine in unilateral DA-depleted rats could be alleviated by unilateral STN DBS [39]. However, in a choice reaction time task, only a few animals remained able to perform the task after the DA depletion (see Fig. 22.4A) and the STN DBS was unable to help those not able to work in the task; in contrast to the spectacular effect of STN DBS in PD patients, the stimulation applied here in the rat could not overcome the profound deficit preventing the animals to perform the task. Interestingly, however, for those able to work in the task, STN HFS alleviated the deficit expressed as a decreased ability of the hemi-parkinsonian rats to initiate a response toward the side contralateral to the DA lesion [39] (see Fig. 22.4B). The conclusion was that STN HFS could be beneficial for the treatment of motor deficit, but non-efficient when the cognitive load was higher, leading to further cognitive studies developed in the next part. Later the same year, Chang et al. [40] showed that STN DBS had a beneficial effect on treadmill walking in parkinsonian rats, while Shi and colleagues [41] have shown a reduced asymmetry when STN HFS was applied in hemi-parkinsonian rats. Gubellini and colleagues [42] also showed recently that STN HFS could restore the use of the contralateral paw that was impaired after 6-OHDA lesion, but was non-efficient to alleviate L-DOPA-induced dyskinesia in hemi-parkinsonian rats, in line with the lesion study published by Marin et al. [43], and possibly because of the well-known effect of STN HFS itself to induce dyskinesia [44].

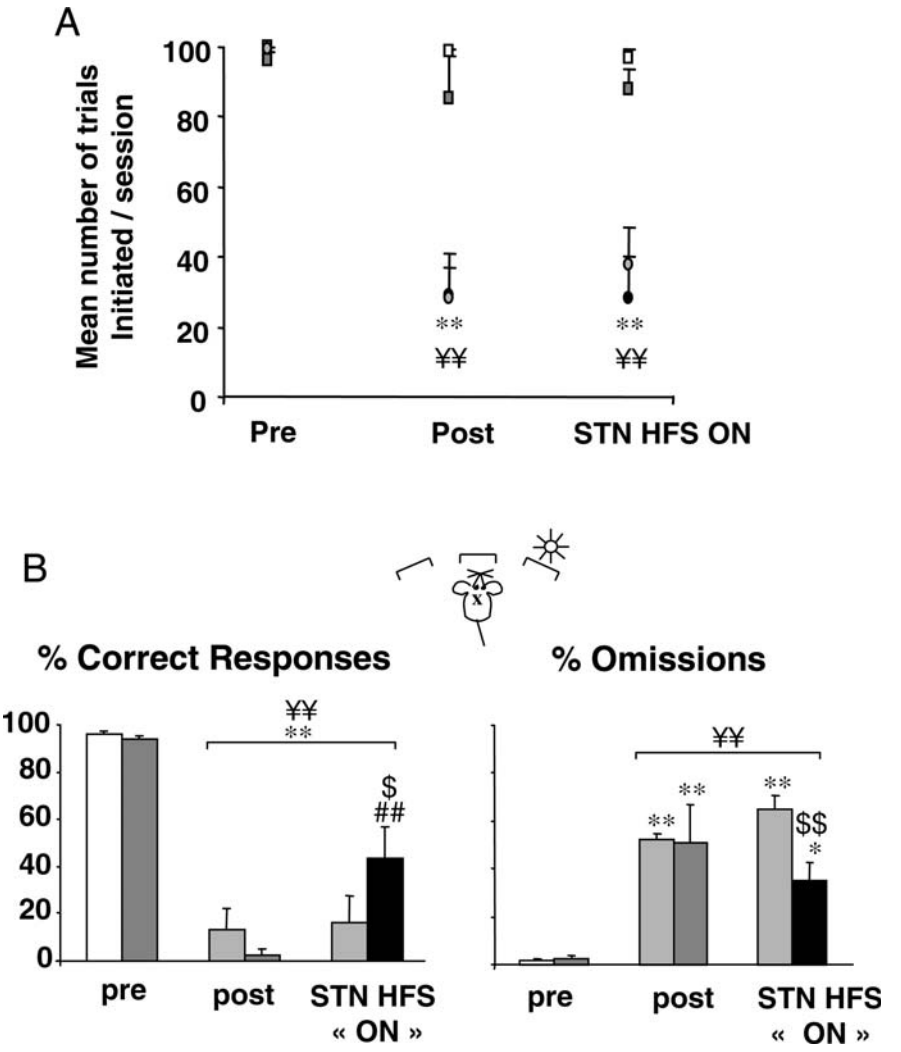

Fig. 22.4 **(A)** Performance in a choice reaction time task after unilateral 6-OHDA infusion in the SNc and unilateral STN high-frequency stimulation [39]. A number of trials were initiated during the various phases of the experiment. **(B)** Performance of the animals still able to work after the DA depletion in terms of accuracy of responses (% correct responses) and omissions toward the contralateral side of the DA lesion before surgery (Pre), after 6-OHDA lesion with stimulation OFF (Post) and after 6-OHDA lesion with the stimulation ON (STN HFS ON) *, **: significatively different from pre-operative performance; ¥, ¥¥: significatively different from sham-control group's performance; $$: significatively different from 6-OHDA non-stimulated group; $p < 0.05$ and 0.01, respectively.

When applied in intact rats, unilateral STN HFS induces contralateral circling behavior that can be reduced by dopaminergic antagonists [45].

The first study testing the effects of bilateral STN HFS was carried out in intact rats and was applied at various parameters in rats performing a reaction time task. STN HFS in that study decreased the premature responses depending on the parameters applied [46]. The same group confirmed that effect on premature responses at different parameters than those alleviating RT deficits in parkinsonian rats [47] and also showed improvement on locomotion [48].

On many aspects of motor behavior, in STN HFS, although not applied always in the same manner (monopolar, bipolar, unilateral, bilateral, individual adjusted parameters or not), the consensus seems to show a beneficial effect on motor deficits induced by parkinsonism. However, the question of a possible detrimental, or at least a lack of, effect on cognitive processes was raised and needed to be further investigated. The animal's data [39, 47] *seem thus to confirm the non-systematic correlation between motor and cognitive effects of STN HFS, as reported in human patients* [49].

Cognitive Behavior

When considering the connectivity of the basal ganglia described as five parallel loops [50], both the GPi and STN belong to each loop and should not therefore be considered only as contributing to motor behavior. As illustrated in Fig. 22.5, the STN receives direct connections from the prefrontal cortex. Therefore, manipulation of the STN should have consequences on frontal functions, as much as it has on motor processes. The STN is also connected more or less directly with structures such as the nucleus accumbens and the ventral pallidum, well known for their involvement in motivational processes. These anatomical considerations lead us to investigate the involvement of the STN in non-motor behavior.

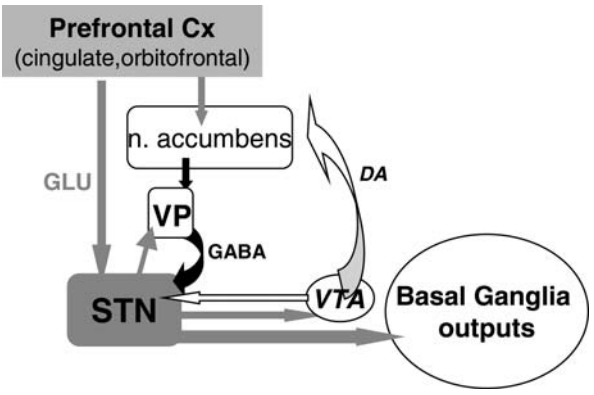

Fig. 22.5 The STN in the limbic loop. The STN receives direct inputs from the prefrontal cortex and indirectly connected with the nucleus accumbens via the ventral pallidum (VP). It receives inputs from the DA nuclei: ventral tegmental area (VTA) and substantia nigra pars compacta

Lesions or STN HFS Data in Monkeys

Only a limited number of groups have studied the effects of STN HFS in monkeys and none have published yet any study investigating its possible effects on cognitive processes. The number of investigations focusing on cognitive processes in human patients has increased in the recent years and might explain why there is little interest for these studies applied to monkeys.

Lesion Data in Rats

There are only a few studies dedicated to the involvement of STN in learning and memory processes. Recently, it has been shown that STN lesions do not affect learning processes seriously, but can affect working memory [51], in line with a former study showing working memory deficits in a choice reaction time task [52]. In our study using a simple reaction time task in 1995, we had suggested that premature responses recorded in the simple reaction task could reflect an attentional impairment [32]. We have used the "5-choice serial reaction time task" in which the animals are trained to wait and detect a brief visual stimulus that can be presented in five possible various positions. The animals have to divide their attention to these five possible holes and then go and respond by a nose-poke in the appropriate hole to obtain a reward in the food magazine and then initiate the next trial (see Fig. 22.6). Using this specific visual attentional task, we have studied the effects of STN lesions first and then of STN lesions combined with a bilateral DA depletion in the dorsal striatum. We first showed that bilateral excitotoxic lesions of the STN induced multiple independent deficits in the task such as impaired accuracy suggestive of an attentional deficit, an increased level of premature responses suggestive of increased impulsivity, an increased level of perseverative responses toward the response locations and the magazine where the animals collect the food reward, suggestive of deficit in response control and increased level of motivation for the reward (see Figs. 22.7 and 22.8) [53]. These results were the first to highlight the involvement of STN in cognitive functions that was strongly suggested by the anatomical data, as previously suggested. They were also replicated after blockade of the GABA receptors into the STN with muscimol [54].

When lesioning the DA inputs to the dorsal striatum, we did not affect dramatically the level of performance in the attentional task; although there was a slight impairment in visual attention, most of the deficits were more motor related (omissions, increased latencies) [55]. Interestingly, when combining this lesion with STN lesions, the performance was further impaired. One of the most striking effects was observed on perseverative responses toward the food magazine, suggesting an increased level of motivation for the reward [55]. In a study using a disconnection between the medial prefrontal cortex and the STN, lesioning the prefrontal cortex on one side and the STN on the other side,

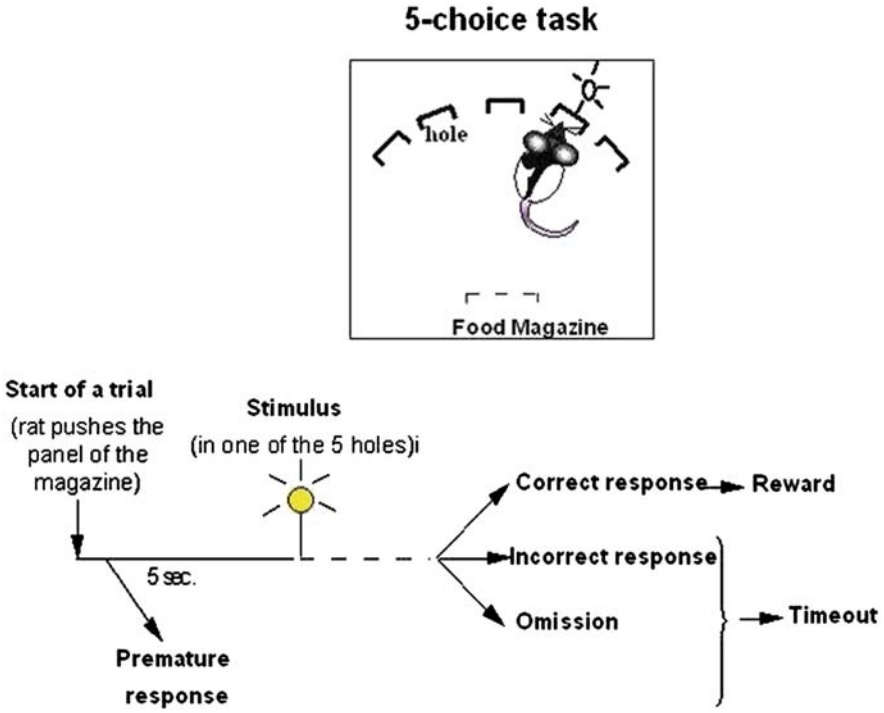

Fig. 22.6 The 5-choice serial reaction time task (5-CSRTT): the rats initiate a trial by a nose-poke in the food magazine. After a 5 s delay, a brief light (500 ms) is presented in one of the five holes. The rats have to detect and respond by a nose-poke in the illuminated hole within 5 s to obtain a reward, collect it in the magazine and then start the next trial. In case of an early response in a hole before the occurrence of the light, the response is recorded as a premature response and punished by a time-out (extinction of the house-light). The same punishment occurs if the rats respond in the wrong hole (incorrect response) or do not respond within 5 s (omission). After the first response has been given, additional nose-pokes in the various holes are recorded as "perseverative responses". Detection of the rats' nose in the food magazine other than the first one after reward delivery is recorded as "perseverative panel pushes" and characterizes inappropriate visits to the magazine

we have given the first evidence of a functional role for the hyperdirect pathway in the attentional and perseverative deficits observed in the attentional task [56]. Further studies have confirmed the role of STN in impulse control. It was indeed shown that STN lesions prevent the animals to be able to stop an ongoing action in a stop signal reaction time task [57]. However, when tested in a behavioral task where the animals are given the choice between a small but immediate reward and a large but delayed reward, the STN-lesioned animals were able to overcome their impulsivity to wait for a bigger reward [58].

These results suggest a specific role of STN in the control of inhibition that can be under the influence of the outcome.

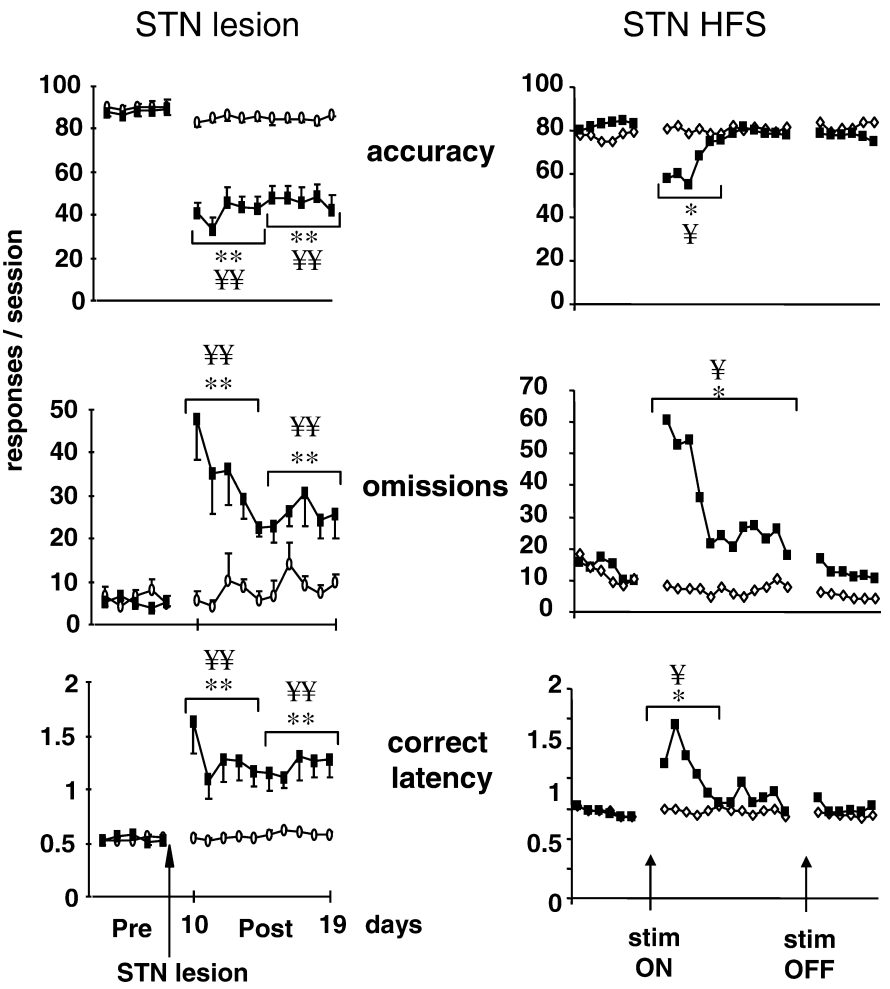

Fig. 22.7 Effects of bilateral lesion and high-frequency stimulation of the STN in the 5-CSRTT [53, 60]. The performance in the 5-CSRTT is illustrated here for accuracy of performance (% of correct responses), number of omissions and mean latency to make correct responses (correct latency) in the sham-operated and STN-lesioned animals (*empty circles* and *plain squares*, respectively; *left panel*) and in the animals subjected to bilateral STN HFS and their control (*plain squares* and *empty circles*, respectively; *right panel*)
*, **: significatively different from pre-operative performance; ¥, ¥¥: significatively different from control group's performance; $p < 0.05$ and 0.01, respectively.

STN HFS Data in Rats

We have previously developed the idea that a premature response in a reaction time task may reflect some cognitive deficit that relates to either an attentional deficit or a deficit in inhibition control. Dopaminergic depletion of the dorsal striatum can sometimes induce an increased number of

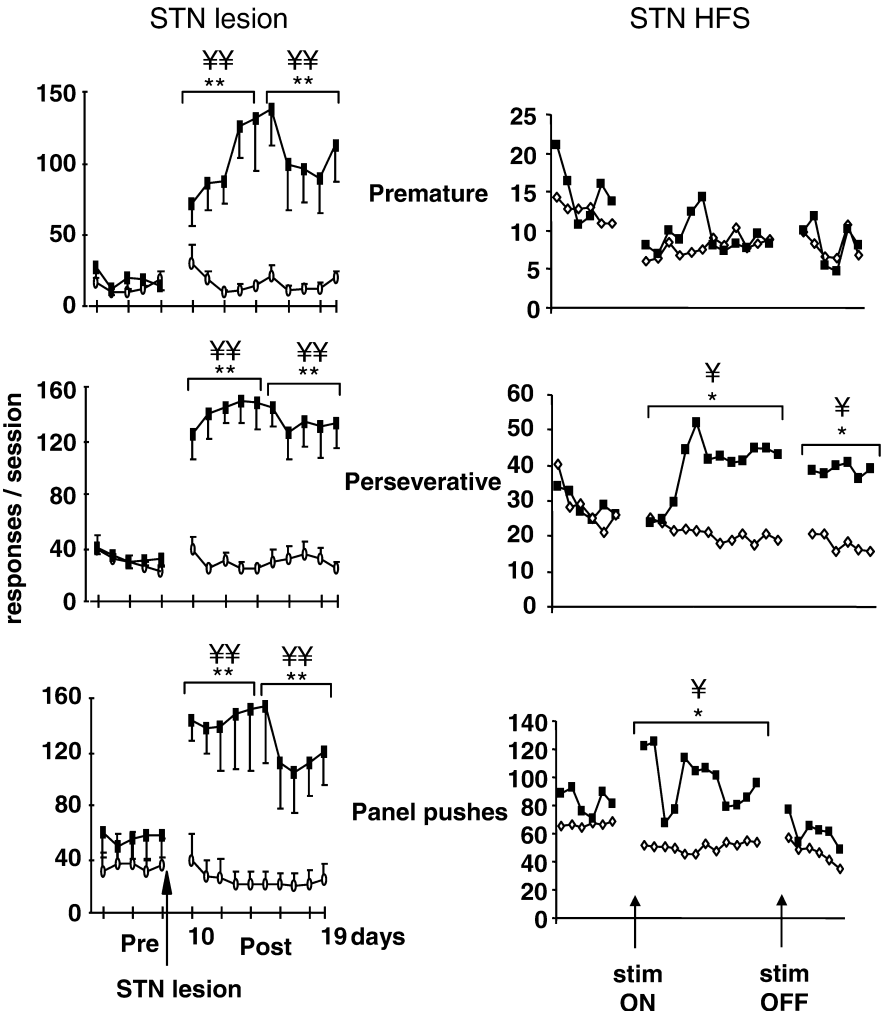

Fig. 22.8 Effects of bilateral lesion and high- frequency stimulation of the STN in the 5-CSRTT [53, 60]. The performance in the 5-CSRTT is illustrated here for premature responses, perseverative responses and perseverative responses into the food magazine (panel pushes) in the sham-operated and STN-lesioned animals (*empty circles* and *plain squares*, respectively; *left panel*) and in the animals subjected to bilateral STN HFS and their control (*plain squares* and *empty circles*, respectively; *right panel*)
*, **: significatively different from pre-operative performance; ¥, ¥¥: significatively different from control group's performance; $p < 0.05$ and 0.01, respectively.

premature responses [59]. Temel and colleagues [47] also reported this type of deficit in parkinsonian rats performing a choice reaction time task, together with increased RT and movement time (MT). Interestingly they have shown that bilateral STN HFS could alleviate the premature-responding deficit at a

very low current intensity (3 μA) than that reducing RT and MT (30 μA) [47]. As mentioned above, this study provides the evidence that cognitive and motor deficits may require a different threshold of HFS to be treated. In intact and parkinsonian rats, we have tested the effects of bilateral STN HFS and could therefore compare them to those induced by bilateral excitotoxic STN lesions in the visual attentional task described above. As illustrated for the intact animals in Figs. 22.7 and 22.8, the effects of STN HFS were slightly different to those induced by STN lesions. Accuracy of performance as well as latency to make a correct response were only transiently affected, while no effect on premature responses could be seen. Interestingly, the perseverative responses on both response location and reward magazine were found, in line with the lesion study [60]. In parkinsonian rats, the subtle deficits recorded in the 5-CSRTT were not further deteriorated by bilateral STN HFS nor alleviated. The most striking effect was observed on the perseverative responses recorded in the food magazine, suggesting that STN HFS increases motivation for the food reward [60].

Motivational Behavior and Psychiatric Models

When investigating the effects of STN manipulation on cognitive functions in the attentional task or in the delay discounting task, we have mentioned a possible increased motivation for the food reward. Indeed, as mentioned earlier, the anatomical connections of STN in the limbic loop suggests that manipulation of the STN should affect motivational processes. We first assessed primary processes of motivation for food to check whether or not STN lesions increase hunger. We have shown that whatever the internal state of the animals (deprived or sated) or the reward (standard animal food, palatable food, alcohol or i.v. injection of cocaine), STN lesions do not affect the consummatory processes [61–63] (Fig. 22.9). When assessing motivation by measures of reactivity to stimuli predicting food, we found that STN lesions increase responses to these stimuli [61]. This result was further confirmed by Uslaner and colleagues [64]. We also showed that STN lesions increase willingness to work on a lever to obtain food pellets and increase the score of preference for an environment previously associated with food [62]. In contrast to these results, we found the opposite effects when the reward was cocaine, highlighting a possible role for STN to modulate the reactivity of the reward system with regard to the nature of the reward involved [62]. In a recent study testing the effects of STN lesions on motivation for alcohol, we have further shown that STN lesions could also affect motivation in an opposite manner depending on the initial preference of the animals for the reward [63]. Although there are no data available of STN manipulation on motivation in animal models of PD,

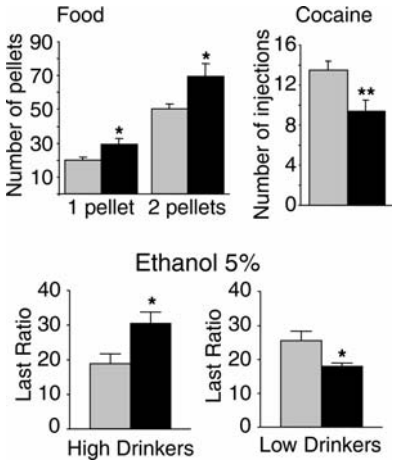

Fig. 22.9 Effects of STN lesions on motivation for food, cocaine and alcohol in high drinkers and low drinkers [62–63]. The performance illustrated here relates to willingness to work for the given reward in a progressive ratio schedule of reinforcement. The animals have to produce an increasing effort to obtain the reward. When the reward was one or two food pellets, STN-lesioned rats (*black bars*) obtained more rewards than the sham-control animals (*dark gray bars*). In contrast, when working for intravenous cocaine infusions (250 μg/infusion), the STN-lesioned rats (*black bar*) worked less than the controls (*gray bar*) and obtained less infusions. When ethanol 5% was the reward, STN-lesioned rats (*black bars*) belonging to the "high drinker" group worked more (higher ratio completed) than the controls (*dark gray bar*), while those belonging to the "low drinker" group worked less than their respective control group (*gray bar*)
*, **: significatively different from the sham-control group; $p < 0.05$ and 0.01, respectively.

these results are in line with some clinical observations in PD patients after STN DBS reporting craving for sweet food in some cases or decreased addictive behavior toward dopaminergic treatment [65].

Finally, although it was not tested in animal models of parkinsonism, it is important to note that recent studies have shown that STN HFS could have a beneficial effect in checking behavior induced by quinpirole, a model that is considered by the authors as a rat model of obsessive compulsive disorder (OCD) [66]. This result is in line with the clinical studies reporting beneficial effects of STN DBS applied in PD patients with OCD [67].

In conclusion for this STN section, it has been shown that most of the effects observed were in line with a beneficial effect of STN inactivation for the treatment of motor symptoms in PD. The rat's studies have raised the issue of non-motor involvement of STN and lead to a better consideration of these aspects in clinical studies. The current interest for motivational and emotional effects of STN DBS in PD patients reflects also the recent interest for these processes in animal models.

Conclusion

In conclusion, this exhaustive review of the literature leads to the following comments:

When investigating the motor behavior, numerous studies carried out in animal models have provided pioneer data supporting the hypothesis that GPi or STN could represent interesting targets for the treatment of parkinsonism. They almost all confirmed the beneficial effects on motor behavior of such a surgical strategy in animals.

However, it is important to note that there are many more studies focusing on STN than on GPi or EP, possibly in line with the predominance of STN surgery in PD over pallidotomies or GPi DBS.

In general, there is a poor investigation of behavioral consequences of HFS in either GPi or STN carried out in monkeys, possibly due to the fact that numerous clinical reports are published every month and might thus reduce the interest proving behavioral efficacy of this surgical strategy. Most of the available studies using HFS in monkeys aimed at understanding the mechanisms of DBS. It would, however, be of great interest to also study the effects on behavior to better understand the functional role of GPi and STN in the basal ganglia, especially regarding non-motor behavior. When it comes to cognitive and motivational processes, only rats' data are available. These studies highlighted the integrative function of the STN, placing it at the interface between motivation and action. There were often a parallel to these findings in clinical observations of PD patients with STN DBS, but further studies in monkeys would be important to perform, especially because they could allow specific investigation of the sub-territories within the STN (limbic, associative and motor areas) that are impossible in the rat given the size of the STN in the rat.

A better knowledge of the possible consequences of GPi or STN inactivation in animals on various types of behavior involving motor, cognitive and motivational processes was important for the treatment of PD patients and has led to a more cautious attitude toward the criteria of selection for surgery. Indeed, with the development of interest in cognitive and psychiatric consequences of STN DBS, the psychiatric examination of the patients has been taken more seriously in order to anticipate and avoid possible side effects of STN DBS.

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Chapter 23
Antidromic Cortical Activity as the Source of Therapeutic Actions of Deep Brain Stimulation

Gordon W. Arbuthnott, Cyril Dejean, and Brian Hyland

Abstract Although deep brain stimulation has been a success in alleviating the motor symptoms of Parkinson's disease the mechanisms of its action remain obscure. In a series of experiments on rats we have documented one novel effect of the stimulation, the antidromic activation of cortical neurons in the motor area. Such antidromic activation also produces a surface-positive wave in the EEG. We have shown a close correlation between the evoked wave in the EEG and the efficacy of the stimulation to release rats from the akinesia that follows dopamine receptor blockade. Such stimulation also prevented the increase in power in the beta band of the EEG that could be seen after dopamine receptor blockade. Along with results from clinical research these results encourage the speculation that the mode of action of DBS might result from direct activation of cortical cells rather than involving any basal ganglia loops.

Introduction

Normal motor control involves a complex interplay between an extensive network of interconnected cortical regions and sub-cortical structures. Prominent among these are the nuclei of the basal ganglia, thought to be involved in learning, initiation, and performance of movement sequences. Disorders of this important system are responsible for several movement disorders, including Parkinson's disease. Projections from the basal ganglia to the cortex via the thalamus are important for regulating cortical output and are strongly implicated in basal ganglia disorders [1–3].

Once the disease has established itself, and the loss of dopamine cells has reached a stage when the administration of L-DOPA no longer can control the

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motor symptoms, invasive surgery becomes an option. Stimulation of the subthalamus has gained an excellent reputation as a palliative measure [4–13] although it does not seem able to change the course of the disease [14]. The method was derived from experiments in which the destruction of the subthalamus reversed the motor symptoms in monkeys treated with the dopamine toxin MPTP. After an initial ballism the monkeys seemed to have recovered their motor control [15–17]. The human equivalent would be a difficult operation but fortunately the stimulation of the nucleus seemed to perform a similar function. The operation is effective but the results of stimulation seem not to be as inhibitory as was expected. Glutamate release increases during stimulation [18]. Dopamine is released by the stimuli in control animals [19]. There is also evidence of GABA release from GP after stimulation [20]. All of these suggest that high-frequency stimulation, far from inhibiting subthalamus, leads to activation of the nucleus and perhaps also to the fibres of passage close to the stimulation site. One set of fibres that seems to have escaped attention until recently is the cortical fibres that are passing close to the subthalamus.

The subthalamic nucleus is situated, in man and animals, cradled in a hammock of the crus cerebri and those myelinated fibres must be the lowest threshold neuronal elements in the region. The same fibres pass close to the globus pallidus sites that are also used in palliative deep brain stimulation. However, even if those are not the target, stimulation in the nucleus might activate the terminals of descending cortico-subthalamic connections whose origin in prefrontal cortical areas suggests another area in which to search for evidence of antidromic activation. Certainly in fMRI studies intended to investigate the role of subthalamic activation in normal control of human movement, subthalamic activation seemed to be causally linked with activation of frontal cortical areas expected to be directly connected with the nucleus [21].

One of the frustrations of the animal research on the substrate of the deep brain stimulation effects has been the plethora of possible candidate pathways involved and the lack of a strategy to choose between them. Results from studies of this question based on predictions of classical basal ganglia network concepts have failed to provide a straightforward account of the mechanism by which changes in basal ganglia activity could produce behavioural benefit. The antidromic activation of cerebral cortex via cortico-STN axons is a novel hypothesis that could solve this longstanding scientific puzzle.

A Direct Test of the Idea in Anaesthetised Animals

Recently in Dieter Jaeger's laboratory in Emory University in Atlanta [22] we looked for activation of the cortex from stimulation in the region of the STN in anaesthetised rats. In one sense the results were not surprising. We could see antidromic activation of cells recorded intracellularly in cortex when we stimulated STN (Fig. 23.1). In another sense they were very surprising because,

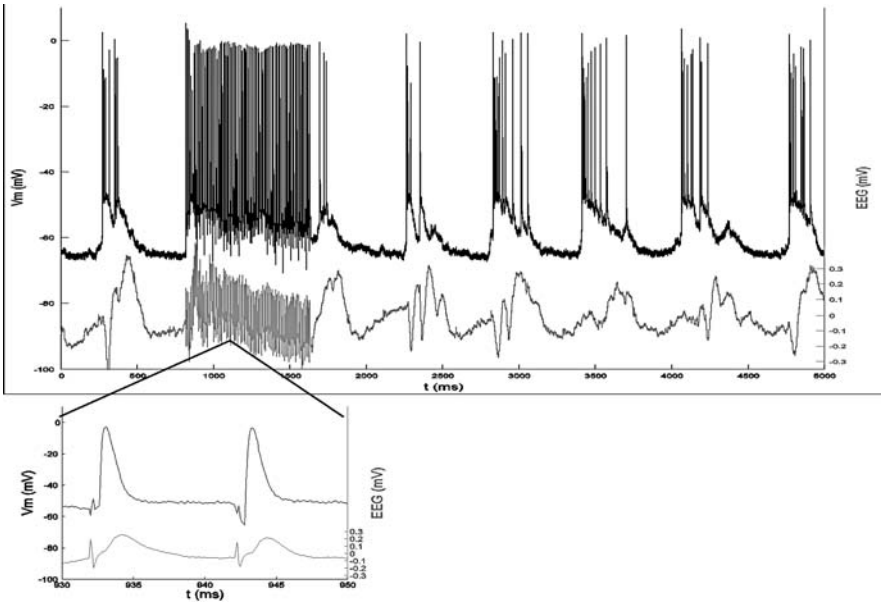

Fig. 23.1 Electrophysiological evidence of antidromic activation in a cortical neuron during 100 Hz stimulation in the ipsilateral subthalamic nucleus in a ketamine-anaesthetised rat. The *black lines* are records of the intracellular potential of a single cortical neuron. The action potentials ride on depolarising plateau potentials that mirror the EEG signal (in *gray*) recorded from a nearby frontal cortical site. The lower pair of records comes from the area of the upper traces indicated and shows the antidromic nature of the action potentials recorded during the train of stimulation at 100 Hz through the subthalamic electrode. The upward deflections on the *gray* trace are field potentials visible only on frontal electrode sites on the EEG traces

whether or not they were antidromically activated, the cells responded to high-frequency stimulation by reducing the membrane oscillations that are commonplace in anaesthetised animals. The stimulation was certainly activating some cortical cells antidromically (about 16% in our sample) but it was having a big effect on the cortical physiology that was reflected in the UP/DOWN transitions of the membrane potentials of the cells. Only layer V cells were antidromically activated but they were present in quite a widespread area around the motor cortex – certainly extending into the adjacent somatosensory area (Fig. 23.2A). The experiments also showed that the activation in cortex was visible on the surface and quite widespread – including the contralateral frontal cortex but not the posterior pole of even the ipsilateral cortex. We also demonstrated that the depth distribution of the response showed an initial activation in layer V with short latency followed by a spread to more dorsal levels later (Fig. 23.2B,C). This depth distribution allowed two conclusions: first, the source of the activation could well be the antidromic activation of corticofugal fibres near the electrode and second, the consequences of direct stimulation of

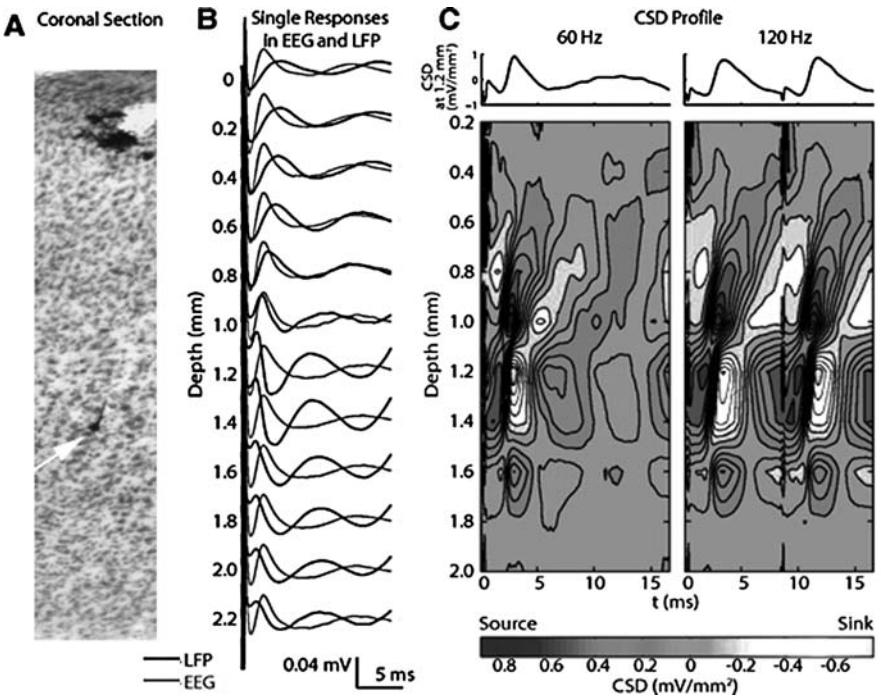

Fig. 23.2 Depth profile of cortical activation after STN stimulation. The coronal section in **A** is aligned with the recordings at various depths in the rat cortex. The *arrow* in **A** indicates a single layer V cell filled with biocytin during intracellular recording in vivo. The cell in this case was antidromically driven from the STN electrode but it is in the most anterior part of the somatosensory cortex as evidenced by the prominent layer 4 visible from 0.6 to 0.8 mm down in cortex. The cell itself is in lower layer V close to the maximum source of the evoked potentials illustrated in **B** and **C**. The set of traces in **B** shows the surface (EEG)-evoked potential in *red* and the simultaneously recorded depth potential in *black*. The current source density plot (CSD) in **C** indicates the potentials plotted against time and depth. The CSD at 1.2 mm depth is shown above for orientation. The *left-hand panel* shows responses to 60 Hz stimulation, while on the *right* the resonance seen following 130 Hz stimulation is shown as deeper source colour and larger source area in the CSD plot
Modified from Li et al. [21] and reproduced with permission.

the cortical surface in patients would not be equivalent to the cortical activation from the descending fibres. This latter conclusion might explain why surface cortical stimulation was less effective than had been hoped [23]. If the activation depth had to be layer V, then the penetration in the deeper human cortex may not have been as effective as in rats – or even as in monkeys – where more hopeful results had been reported [24].

The surface-evoked potential on cortex had another consequence. Complicated electrophysiology was not necessary to record the cortical-evoked potential and it was visible from many areas. Could such a potential serve to identify cortical activation and could we then correlate the activation in cortex with

some measure of outcome at a behavioural level? Two results from the clinic gave us hope that we were on the right track. First, Ashby [25] had seen similar cortical-positive responses at short latency in the EEG of patients when stimulated through a DBS electrode in STN. Second, there were a set of reports that patients who had already had a pallidotomy and who were still in trouble with parkinsonian signs had benefited from stimulation through a subsequent STN implant [26–28]. So STN stimulation does not need an intact basal ganglia for the effect.

Some Experiments on Alert Animals

We then needed an experiment that would assess the link between the cortical correlates of STN-DBS and the potency of the latter to counteract parkinsonian symptoms. For that purpose at the University of Otago in Dunedin, we recorded cortical EEG in an acute rat model of Parkinson's disease (combined D1 and D2 receptor blockade) that received electrical stimulation through deep brain electrodes placed in the STN. As expected we recorded the short latency positive surface potential that was shown to reflect antidromic invasion of cortical neurons [22]. The animal behaviour responded positively to high-frequency stimulation and we observed a spectacular reversal of the drug-induced parkinsonian-like akinesia when high-frequency stimulation was applied at a rate of 130 Hz (Fig. 23.3) in a replication of recent results obtained in two other labs [29, 30]. Alongside the strong akinesia the dopamine antagonists injection was also followed quickly by an increase in beta oscillations

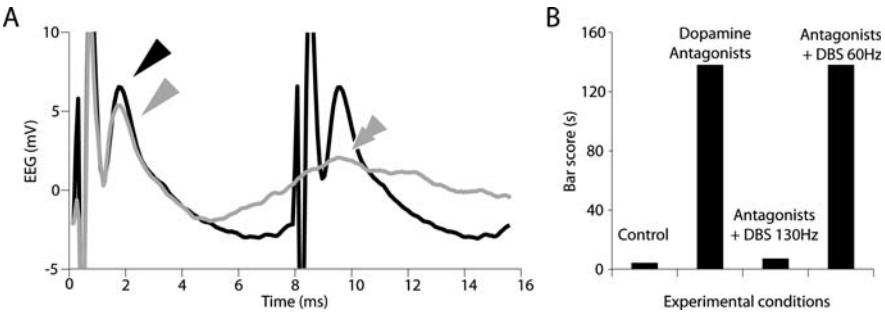

Fig. 23.3 Evoked potentials in the cortex following STN-DBS and associated behavioural improvement in the alert rat. A The picture shows the average of 150 evoked potentials in the cortex of an animal stimulated in the STN at 130 Hz (*black trace*) and 60 Hz (*grey trace*). At 60 Hz, STN stimulation elicits a polyphasic response in the cortex with an early (*arrow*) and a late (*double arrow*) positive component. At 130 Hz both the late and the early components are superimposed resulting in an increase in the amplitude of the cortical response (*black arrow*). **B** The bar test score measures the extent of rigid akinesia in animals. In the same animal as in **A**, dopamine antagonists induce a strong impairment that is completely reversed by 130 Hz STN-DBS but not 60 Hz

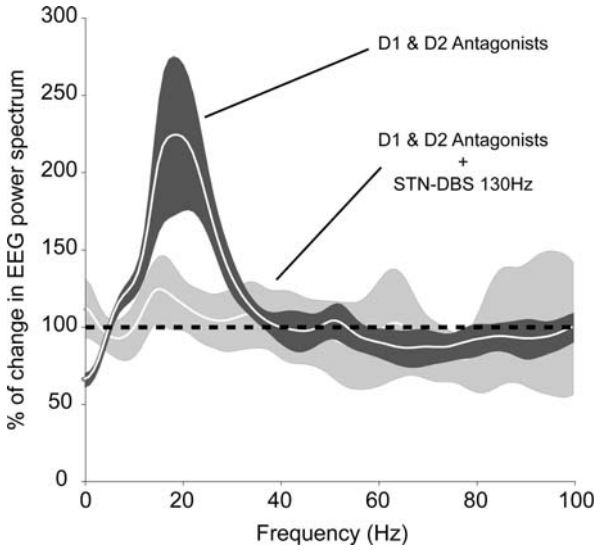

Fig. 23.4 Effect of STN-DBS on change in frequency components of EEG induced by D1/D2 antagonists. The graph displays an example of the changes in power spectral density after combined injection of D1 and D2 antagonists with or without STN-DBS. The percentage of change compared to baseline is averaged across 10 min postinjection. The antagonists elicit a strong increase in oscillation between 12 and 30 Hz. In experiment where STN-DBS is applied at 130 Hz, this increase is completely counteracted

(Fig. 23.4) often suggested to be a marker of Parkinson's disease. Such low-frequency oscillations are seen in the activity of basal ganglia and cortex of parkinsonian patients and animal models [31–38]. Interestingly, this increase in our rats was totally abolished by the stimulation (Fig. 23.4). Normalisation of the behaviour was dependent on the stimulation producing an evoked potential, and scaled with the potential amplitude. Across all experiments (Fig. 23.5) there was a strong inverse relationship between the level of akinesia, as assessed with a bar test, and the amplitude of the evoked potential. Thus the positive behavioural effect of the STN-DBS is a function of its impact on the cortex.

Resonance as the Explanation for Frequency Dependence

While 130 Hz stimulation frequency proved to be extremely efficacious, 60 Hz stimulation resulted in little or no improvement of the symptoms in the same animals (Fig. 23.3). This observation is also commonly reported in patients and other animal models of Parkinson's disease. Interestingly both the studies in Atlanta and Dunedin showed that 130 and 60 Hz stimulation frequencies were associated with different impacts on the cortex. At 60 Hz or lower frequencies, the electrical stimulation of the STN evoked polyphasic responses in the cortex (Fig. 23.3) with notably an early peak at a latency of about 2 ms and a slower

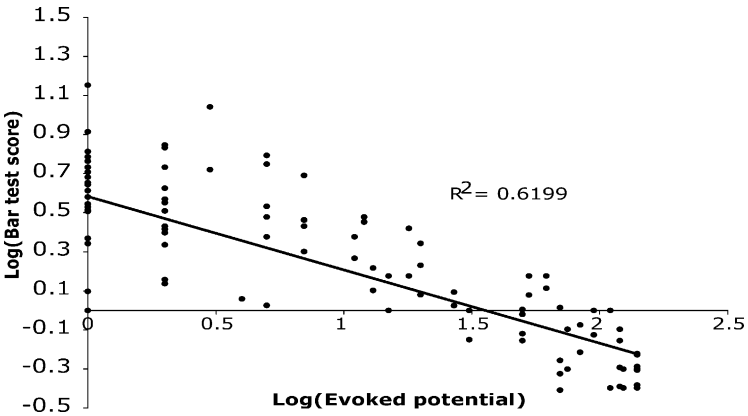

Fig. 23.5 Relief from parkinsonian symptoms is correlated with the effect of STN-DBS on amplitude of cortical-evoked potential. Points show the bar test score (rigid akinesia) as a function of the peak amplitude of the evoked response evoked in the cortex on a double logarithmic scale for all 86 recording sessions in which bar test score and EEG were both recorded. Data were obtained from nine animals (18 hemispheres). The linear correlation shows that akinesia is decreasing as the amplitude of the cortical response grows

and smaller late peak at 9–12 ms. At high frequencies such as 130 Hz, the late component of the response from one stimulation is still occurring when the next arrives, allowing superimposition that can be more than just additive. The effect of STN-DBS at 130 Hz thus seems potentiated compared to 60 Hz thanks to resonating properties of the cortical network. Moreover we also showed that at 130 Hz bilateral stimulation is more efficient than unilateral stimulation in activating the cortex. This might reflect additional inter-hemispheric resonance with responses carried by the callosal connections prominent in frontal cortical areas (Fig. 23.6). This resonance effect could be at the origin of the discrepancy of behavioural effect between 130 and 60 Hz stimulation. First, locking cortical networks on the 130 Hz rhythm could serve to keep the system away from potentially pathological rhythms such as beta oscillations. Second, the resonance effect sustains activation in the cortex, the amplitude of which is closely related to the behavioural improvement.

Thus we have strong, but circumstantial, evidence that the improvement in behaviour – and perhaps the therapeutic efficacy of STN-DBS – is closely associated with this cortical synchronising effect of the stimulation. Recovery from akinesia is, at very least, closely related to the size of the cortical-evoked response. The early-evoked response in cortex response is most likely, because of its short latency, to be a consequence of antidromic driving of layer V cells in frontal and motor areas of cortex. Such stimulation of the cortical input and output stations of the basal ganglia "motor loop" might have an important role to play in reducing the synchronous activation of basal ganglia structures that has been suggested to underlie the motor incapacity in patients and in MPTP-treated monkeys [32,

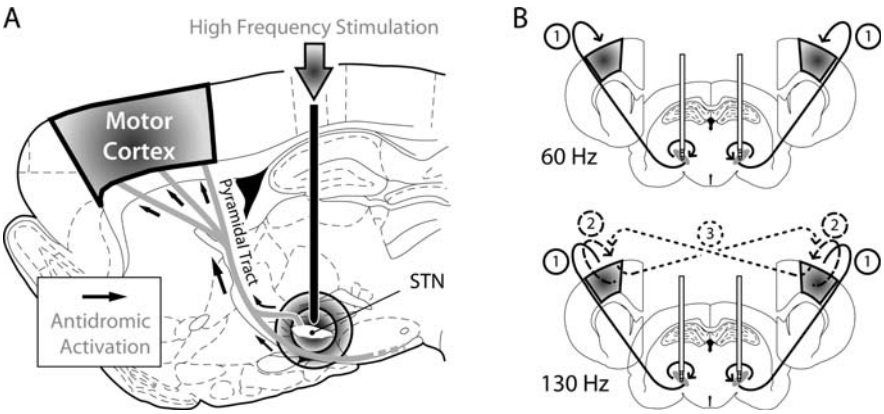

Fig. 23.6 Resonance effects of high-frequency stimulation. The diagram in **A** represents the likely route for activation of the layer V-evoked response from subthalamic stimulation. In **B** are shown the lack of resonance at 60 Hz when the response invades the frontal areas, but the late effects of the stimulus is over before the next pulse. At 130 Hz in contrast, both the local effects in motor areas (2) and the crossed (likely callosal) pathways (3) all resonate together producing the observed increments in the evoked responses. We hypothesise that the resonance produced overcomes any basal ganglia beta frequency driving and so releases the cortex to maintain more normal motor responses during stimulation

39–41]. Rhythmic drives from the basal ganglia structures might entrain the cortex to their own beta frequency oscillations. The cortical driving enforced by the new resonance created by antidromic activation might break up such pallidal rhythms. Thus the motor command system might once again activate normal movements via the damaged indirect route through the basal ganglia maze.

Some Suggestions for Future Work

How do we get the definitive proof that this is the source of the healing? There seem to be several tests of the idea. First any stimulation that entrains large areas of cortex should do the same trick. It may be difficult to use the pyramid as stimulation site because of more direct routes to the muscles from there. Perhaps a pontine site would do similar things? On the other hand interrupting the basal ganglia loops should not block the effect of stimulation while cortical damage should abolish the effect. Of course we should try both tactics to finally pin down the source of the therapeutic effect of deep brain stimulation – though as a prominent neurologist said recently at an international conference "Whatever the source turns out to be, the therapeutic benefit is not in doubt, so the procedure is certain to be used more widely!". There is a challenge there as well as a warning!

Acknowledgments The experiments reported here were supported by the Michael J Fox Foundation and Neurological Foundation of New Zealand.

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Chapter 24
Cell-Based Replacement Therapies for Parkinson's Disease

Emilio Fernández-Espejo and Isabel Liste

Introduction

Parkinson's disease (PD) is caused by the loss of dopaminergic neurons of substantia nigra. The motor symptoms of PD (tremor, bradykinesia, akinesia, rigidity, and disturbances of gait and posture) are due to the progressive loss of these dopaminergic neurons that project to the striatum. The most prevalent therapy is oral levodopa (in combination with compounds that enhance its bioavailability), but levodopa is not efficacious after several years of treatment. An alternative therapy to levodopa is the use of intrastriatal grafts of cells capable of ameliorating functional deficits of PD. Since the 1980s, grafting procedures have been extensively tested in animal models of PD, before their clinical application [1–9]. Transplantation is considered as a promising treatment for human Parkinson's disease, and grafting procedures performed as clinical therapy for PD could be classified in two main groups: (i) grafts devoted to the restoration of the dopamine levels in the denervated striatum by using dopamine-secreting cells (i.e., fetal or embryonic dopaminergic cells, engineered dopaminergic stem-derived cells, retinal pigment epithelial cells, etc.), or cultured sympathetic neurons that convert exogenous levodopa in dopamine, and (ii) dopaminotrophic grafting procedures directed to provide a trophic support for the remaining host nigrostriatal dopaminergic neurons, in order to stop neuronal death or to restore the striatal dopaminergic circuit. Another plausible form of cell therapy is the activation of pre-existing neuronal precursors located in neurogenic centers that could migrate and differentiate to replace destroyed cells in some parts of the brain. This possibility is speculative at present and based solely on preliminary experimental obseravtions.

It is reasonable to think that, since levodopa significantly ameliorate functional deficits in PD, grafting dopamine-secreting cells for providing a constant

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source of dopamine should be as efficient as systemic levodopa therapy. In this context, intrastriatal grafting of dopamine-secreting cells obtained from neural or chromaffin tissues, such as fetal mesencephalon, retinal pigment epithelium, adrenal medulla and carotid body, has been reported to ameliorate functional deficits in animal models of Parkinson's disease [10–14]. However, its clinical use is still restricted to few cases, it is under discussion or it has been abandoned. As a general overview, clinical trials have shown that mesencephalic dopamine neurons obtained from human embryo cadavers can survive and function in the brain of patients with PD [15, 16], and long-lasting functional improvement has been reported in many grafted patients. However, major limiting factors regarding fetal mesencephalic cells are the ethical, practical and safety issues associated with tissue derived from aborted human fetuses, and the difficulty in obtaining sufficient viable embryonic mesencephalic tissue [17, 18]. Stem cell therapy appears to be a good candidate for transplantation in PD and could overcome the problem of the limited availability of human fetal neural tissue and the low survival rate of fetal dopamine neurons [19]. On the other hand, adrenal cells were the first dopamine-secreting cells to be investigated, but these cells are no longer used because their long-term survival is very poor in the brain (even after autotransplants), and beneficial effects are transient either in Parkinson's patients or in animals [20, 21]. As regards retinal cells, implants of human retinal pigment epithelial cells attached to gelatin microcarriers appear to be safe and well tolerated, and they partially improved motor symptoms in patients with Parkinson disease [22]. It seems that these cells can act as dopamine "minipumps" leading to a partial improvement of motor deficits, although their long-term integration and functionality have yet to be demonstrated. Carotid body cells were shown to be effective in rodent and monkey models of PD [7, 13, 23], but the clinical efficacy of glomus cell transplants should be improved. A major advantage of glomus cells, like adrenal ones, is that they can be used for autotransplantation, thereby avoiding tissue rejection and the need for immunosupression therapy. However, their clinical efficacy is poor in advanced Parkinson's disease in humans where the carotid body is known to be also affected by dopaminergic cell degeneration. This limitation could be overcome by using an unlimited source of glomus cells, and recently glial sustentacular cells of the carotid body have been demonstrated to behave as stem cells for glomus type I cells [24]. Stem cell-derived glomus cells are dopaminergics and produce glial cell line-derived neurotrophic factor, hence carotid body stem cells could be potentially useful for antiparkinsonian cell therapy.

Another cell therapy strategy, as mentioned, is to introduce potentially neuroprotective molecules to prevent cell death, slow down the process or even stimulate regeneration in the damaged dopaminergic nigrostriatal system by using trophic factor-releasing cells or viral vectors. These cells and viral vectors would exert a "dopaminotrophic" action, and if grafted cell vectors give rise to neuritic processes extending far away from the site of implantation, the trophic action would be widespread and likely more effective [17]. This is a quite different approach to grafting dopamine-secreting cells, since more extensive

reinnervation and tissue regeneration is searched, rather than focalized enhancement of dopamine release. Among dopaminotrophic factors, glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) have potent in vivo effects [20, 25, 26, 27]. Apart from being potentially pharmacological tools, these factors have also been administered into the brain. Promising results have been obtained with fibroblasts and astrocytes engineered to secrete GDNF [28, 29] or BDNF [30, 31] and with viral vectors expressing GDNF [32, 33]. In vivo neuroprotective effects have been reported to be in the order of 40–70% rescue of nigral dopamine cells. Transforming growth factor-β_1 (TGF-β_1) is another trophic factor that has also been reported to protect dopamine cells in vitro [34], and it is a cofactor that potentiates the neurotrophic actions of GDNF in vitro and in vivo [35, 36]. In this context, the antiparkinsonian effects of cell grafts of the sympathoadrenal lineage are attributed not only to dopamine release from grafted cells but also to dopaminergic reinnervation of the denervated striatum [11, 13, 14, 17, 37], and chromaffin cells are known to express and release GDNF and TGF-β_1 [38–40]. Grafted adrenal cells seem to work as dopaminotrophic cells that induce sprouting of surviving dopamine nigral cell, and reinnervation of damaged striatum, although cell survival is low. Recently, extra-adrenal chromaffin cells have been reported to exert strong neurotrophic effects based on the delivery of GDNF and TGF-β_1, and unlike adrenal chromaffin cells a significant proportion of extra-adrenal cells show long-term survival after transplantation [41, 42]. Finally, grafted carotid body dopamine cells (type I cells) have been observed to exert an added neurorestorative effect, that it has been related to the trophic action of GDNF, because type I glomus cells express and release this trophic factor [43].

Grafts Based on Cells of the Sympathoadrenal Cell Lineage

Sympathoadrenal (SA) cell lineage encompasses sympathetic neurons, small intensely fluorescent (SIF) cells of sympathetic ganglia and adrenal medulla, chromaffin cells of adrenal medulla and extra-adrenal paraganglia, and serotonergic enteric neurons. SA cells derive from a common progenitor of the neural crest (for a review see [44, 45]). Most SA cells are "adrenergics", which means that they synthesize either adrenaline (80% of adrenal chromaffin cells) or noradrenaline (20% of adrenal chromaffin cells, all extra-adrenal cells and sympathetic neurons, and 20% of SIF cells) [46].

The ideal donor tissue for transplantation is the patient's own tissue, which avoids graft rejection and the need for immunosuppressant therapy. In this way, almost all SA cell types have been transplanted in animal models of PD and some of them have been also tested in human as autologous cell-replacement therapy for PD. Therapeutic effect exerted by grafted SA cells in PD acts in two ways: (i) an important restorative neurotrophic action on damaged host dopaminergic tissue through the delivery of dopaminotrophic factors such as GDNF

and TGF-βs, which protect dopaminergic neurons from degeneration [25–27, 38, 39], and (ii) a partial recovery of dopamine levels by the release from the graft of a certain amount of dopamine (mainly adrenal cells), even though only minute amount of dopamine are released from most SA cells [8, 17, 42, 47, 48]. The greatest handicap of using SA cells for grafting is their poor survival in the brain, even though some types of SA cell grafts such as extra-adrenal chromaffin cells appear to present a better survival rate, as it is discussed below.

Sympathetic Neurons

Several research groups have grafted superior cervical ganglion (SCG) neurons in animal models of PD with widely varying results. SCG neurons release NA and express "dopaminotrophic" factors and other neuroprotective agents such as neuropeptides and cytokines [49]. Stenevi et al. [50] reported a very poor survival of striatum-transplanted sympathetic neurons, except for those grafts placed near the choroidal fissure. In these cases the revascularization of the transplant from the choroid plexus vessels enhances graft survival. Thus, the close relationship between a graft survival and an efficient revascularization from the surrounding tissue strongly suggests that adequate oxygen supply is a key factor for graft survival. Grafted cells next to the choroidal plexus give rise to numerous fibers or neurites spreading into host brain. This interesting phenomenon has been frequently observed in grafted sympathetic SA cells and other neural-crest derived cells (i.e., carotid body cells), and opens the possibility that host striatum neurons and grafted cells could establish synaptic contacts that might regulate graft dopamine release. However, in contrast to transplanted fetal dopaminergic neurons [51, 52], the establishment of host graft synapses has not been yet demonstrated in sympathetic SA cell grafts.

Transplanted sympathetic neurons into the host striatum far away from the choroidal plexus fail to survive, and even NGF preincubation of grafted neurons is unable to improve cell survival [50]. Thus, the main limitation of SCG cells graft into adult brain is the massive cell death after transplantation into the striatum [53]. This handicap seems to have been solved by using cultured sympathetic neurons for grafting. In this case, grafts long-term survival depends on the time of maintaining cells in culture, being maximal with 2-week cultured cells [54]. Grafts of these cultured cells significantly reduced the rotational behavior after apomorphine administration 12 weeks after transplantation [54]. Because of the apparently beneficial effects of cultured sympathetic cells in animal PD models, autologous SCG transplantation in PD patients has been tested [55]. In five grafted PD patients the on-period induced by levodopa was improved [55], and the graft-mediated effect is detectable during a follow-up period of at least 1 year post-grafting. Although SCG grafted cells are noradrenergic, their beneficial effect could be explained by their capability to convert exogenous levodopa to dopamine [55, 56]. Thus, cultured sympathetic neurons grafts induce a partial symptomatic relief in PD patients and reduce the need for

levodopa medication. Grafted sympathetic neurons can provide a site for both the conversion of exogenous levodopa to dopamine and the storage of the synthesized dopamine in dopamine-denervated striatum because the beneficial effects are attenuated when given reserpine treatment [57]. Therefore, sympathetic ganglia neurons can be used as donor tissue for autologus transplantation in PD, but further investigation is required to improve cell survival, the main disadvantage for the clinical application of this cell substitution technique.

Adrenal Chromaffin Cells

Adrenal chromaffin cells were the first non-neuronal cells grafted in animal models of PD. These chromaffin cells constitute the medulla of the adrenal gland. From a clinical point of view, they have the advantage that they can be obtained from one of the patient's own adrenal glands. Autologous adrenal cells transplanted into the denervated striatum exerted some beneficial effects in animal models of PD [1, 3], and this was also the case for the first reported grafting in PD patients [58]. Since the proportion of dopaminergic cells is very low in transplanted donor tissue (only 1% of the entire adrenal chromaffin cells population releases dopamine), the graft functional effects could not be exclusively accounted for by dopamine release. In fact, as it is the case of other members of the SA cell lineage, the neurotrophic effect of chromaffin cells leading to sprouting of host dopaminergic fibers was proposed as responsible for PD symptoms amelioration [49]. Indeed the sprouting of host dopaminergic fibers was first observed after adrenal chromaffin grafts, because Bohn and associates [3] demonstrated a robust increase in TH-ir within the striatum of MPTP-treated mice despite poor adrenal graft survival.

However, the initial good results were not confirmed by other authors [59], and it is currently accepted that survival of adrenal medulla grafts is low in animal models of PD [5], and extremely low after grafting in PD patients [56], which show only transient functional amelioration after grafting [60, 61]. Although many benefits of adrenal medullary grafting were reported, the most consistent improvements were increased "on" time and improvement in motor function, but these effects were lost after 1–2 years. Transient complications were frequent (American Association of Neurological Surgeons General Registry for Adrenal and Fetal Transplantation – GRAFT Project). Thus in an evaluation of over 126 patients operated in the United States, an average 19% had medical complications, 9% had abdominal complications and 13% had intracranial complications [62]. Histological studies of long-term grafts experiments give the explanation. Grafts became necrotic and were surrounded by a perigraft halo [3, 63–65] of microglia and macrophages which persist for many months after grafting, limiting thus the trophic action of the grafts [66]. Therefore, long-term survival and functional efficacy of adrenal chromaffin cells grafts are very poor in the brain, either in experimental PD models or in PD patients, and this approach is no longer pursued clinically (see Table 24.1).

Table 24.1 Features of cell grafts used for antiparkinsonian treatment

Type of cell	Dopamine secretion	Neurite outgrowth from graft	Trophic striatal reinnervation	Long-term survival rate	Secretion of trophic factors	Clinical efficacy
Sympathetic neurons	Minute	Yes (only non-cultured cells)	Scarce	0% (solid tissue) 1–3% (2-week cultured cells)	Unknown	Partial, reduce the need of levodopa [55, 56]
Adrenal chromaffin cells	Minute	Low	Yes	0%	Secrete GDNF	Poor and limited
Extra-adrenal chromaffin cells	Minute	Low	Yes	1% (cell aggregates)	Secrete GDNF and TGF-beta1	Non tested
Carotid body glomus cells	Yes	Yes	Yes	High (cell aggregates) **	Secrete GDNF	Limited #
Fetal mesencephalic cells	Yes	Yes, abundant	Scarce	3–5%	Scarce	Acceptable *
Stem cell-derived dopamine cells	Yes	Unknown	Scarce	Yes, but not quantified	Scarce	Non-tested
TH- and GDNF-expressing genetically engineered astrocytes	Yes	No	Yes	Good	Secrete GDNF	Non-tested
GDNF- and BDNF-expressing genetically engineered fibroblasts	No	No	Yes	Low	Secrete GDNF or BDNF	Non-tested
Retinal pigmental epithelial cells	Yes	Low	No	0% (they must be attached to microcarriers for good survival)	Secrete PEDF and other trophic factors ##	Non-tested ###

*Some disadvantages are the difficulty in obtaining sufficient viable embryonic mesencephalic tissue and that some patients have been reported to present disabling dyskinesias [103]; ** long-term graft survival has been reported in most of transplanted animals, without estimating the number of surviving grafted cells [23]; # limited efficacy of autologous transplants has been explained by PD-related deterioration of old carotid body glomus cells and the reduction in chromaffin cell number with age [95]; ## according to the authors, pigment-derived epithelial factor (PEDF) accounted for only a portion of the neurotrophic effect indicating that differentiation increased the production of other trophic factors as well [22]; ### a randomized, double-blind, placebo-controlled study has been initiated.

Since adrenal chromafin graft survival and differentiation into a neuronal phenotype are enhanced by treating the grafts with nerve growth factor (NGF), as reported by Strömberg and associates [67], co-graftings of adrenal medulla tissue with peripheral nerve as a source of NGF have been done, and a moderate improvement has been reported in some cases [68–71].

Extra-Adrenal Chromaffin Cells

Extra-adrenal chromaffin cells are currently used for grafting in animal models of PD in the first author's laboratory [41, 42, 77] and others [72]. Extra-adrenal chromaffin cells are located in extra-adrenal paraganglia which derive from the same population of SA cells of the ganglion primordia that migrate and invade the adrenal primordium, but these cells migrate outside the adrenal primordium and invade ectopic locations. Extra-adrenal paraganglia are located adjacent to organs near the adrenal gland (mainly kidneys), on the abdominal sympathetic region (solar plexus), next to the genitals glands and on the low abdominal aorta (the Zuckerkandl's paraganglion or ZP). This paraganglion was discovered and described by Emil Zuckerkandl in 1901 [73]. Paraganglia are mostly constituted by mesenchyma and chromaffin cells (see Fig. 24.1). Chromaffin cells aggregate in fascicles surrounded by mesenchyma, with the appearance of "cell nests" on coronal sections. This arrangement of chromaffin cells was originally described by Kohn in 1903, who named fascicles and nests as "Zellsträngen" and "Zellballen", respectively [74, cited in 75 and 76].

The Zuckerkandl's paraganglion is the main extra-adrenal paraganglion and is found to be located lying on the abdominal aorta, near the emergence of iliac

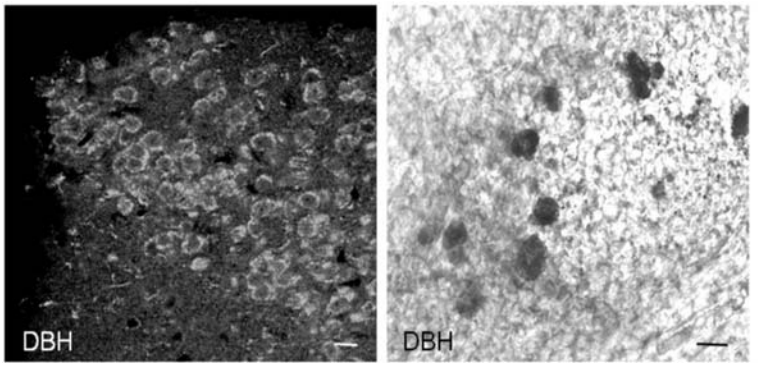

Fig. 24.1 *Left*: Immunofluorescence micrograph of a piece of the Zuckerkandl's paraganglion used for transplantation, showing a clustrer of rounded DBH+ chromaffin cells surrounded by mesenchyma. *Right*: A group of surviving grafted chromaffin cells expressing DBH, three months post-graftin. Bars 15 μm

arteries. ZP can be easily removed from its location [41, 42], and the experiments indicate that, after transplantation of ZP cells in the form of cell aggregates, parkinsonian rats (6-OHDA model) show a long-term behavioral improvement manifested by a progressive and sustained reduction of several motor and sensorimotor parkinsonian deficits [41, 42, 77]. These functional effects are related to survival of around 1% of grafted cells at 5 months after grafting, a remarkable finding that indicates that extra-adrenal chromaffin cells unlike adrenal ones survive for a long period of time. This survival rate is slightly lower to that reported for fetal mesencephalic cells grafts [78, 79], and similar to that of cultured sympathetic cell grafts, another cell tissue of the sympathoadrenal lineage [55, 56]. The placement of ZP within brain parenchyma does not induce changes in the phenotype of ZP cells, which different to other grafted neural-crest-derived cells (i.e., carotid body cells, [23]) do not develop neurite-like prolongations. Immunohistochemical analyses reveal the presence of TH+, DBH+ (see Figs. 24.1 and 24.2) and CgA+ cells inside grafts [42, 77], and TH+ density of host striatum is significantly augmented after grafting (Fig. 24.2); TH-ir being accompanied by a reliable increase (even though two times lower than that of naïve control animals) of striatal dopamine content [41]. Hence, this partial dopamine levels' restitution after ZP grafting explains the behavioral improvements obtained in parkinsonian rats, which are directly related with the recovery of the dopaminergic tone of dorsal striatum [2, 4, 80]. However, the increase of striatal dopamine content after grafting cannot be explained by dopamine ZP cells release, because grafted cells are noradrenergic, a small amount of dopamine can be detected in ZP tissue, and only minute amounts of dopamine can be released from extra-adrenal noradrenergic chromaffin cells [8, 42, 47, 48].

The recovery of the dopaminergic tone after grafting can be related to striatal reinnervation due to sprouting of spared host dopaminergic fibers. ZP-grafted cells express GDNF and TGF-β_1, and significant levels of these neurotrophic factors are detected in the striatal tissue [41]. GDNF and TGF-β_1 protect dopaminergic neurons from degeneration [20, 81–86]. GDNF induces sprouting of dopamine axons, and antisense inhibition of GDNF expression reduces dopamine axon sprouting on striatal injury [87]. GDNF can also act through a reduction of degeneration of dopamine fibers, a process that is known to be regulated by intracellular pathways such as the ubiquitin–proteasome system. Inhibition of this system enhances dopamine neuron degeneration [88] and GDNF protects against proteasome inhibition-induced dopamine neuron degeneration by suppression of endoplasmic reticulum stress and caspase-3 activation [89]. Regarding TGF-β_1, this factor is known to protect dopaminergic neurons when delivered in vitro [34] and also acts as a cofactor which potentiates the neurotrophic actions of GDNF in vitro and in vivo [35, 36]. Recently it has been proposed that combined actions of both factors are necessary for full neurotrophic effects and that TGF-β_1 may be essential for permitting exogenous GDNF to act as a neuroprotective factor [90]. The molecular mechanisms underlying this action are not well known, but TGF-β_1 and GDNF could

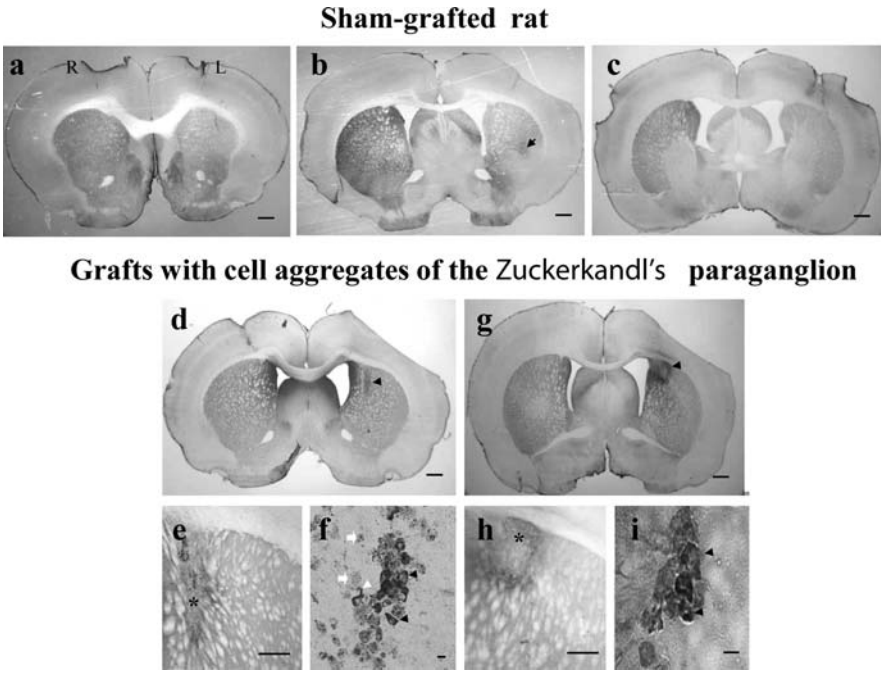

Fig. 24.2 Morphological features of striatal regions and grafts in hemiparkinsonian rats subjected to grafting with cells of the Zuckerkandl's paraganglion or sham-grafted animals (**a**-**c**) Sham-grafted rats showed a lower TH-ir density in the left striatum, in the three coordinated corresponding to 6-OHDA infusions (from *left* to *right*). Only a limited TH+ halo around the grafting site can be observed (*arrow*) (**d**-**i**). In rats with grafts of ZP cell aggregates, a net recovery of TH-ir in the left striatal region encompassing the graft was observed (**d**, **e**, **g**, **h**, *black arrow head*) as compared with the contralateral striatum (compare **d** and **b**), and perigraft TH-ir was even denser. Grafts (*asterisks*) contained surviving TH+ cells (**e**, **f**, **h**, **i**) with a chromaffin-like morophology and mostly associated in clusters (**f**, **l**, *arrow heads*), as it is their arrangement in vivo. Some chromaffin cells showed neuritic processes (**f**, *white arrow* head). Infiltrative cells were also observed (**f**, *white arrows*). Bars (**a**, **e**, **g**) 1 mm; (**e**, **h**) 500 μm; (**f**, **i**) 15 μm (modified from 42)

cooperate during several crucial steps of signal transduction, including GFRα1 receptor membrane localization [91].

Recovery of TH activity in the host striatum of rats grafted with ZP cells could also be related to the action of GDNF because striatal GDNF administration is known to increase TH phosphorylation and hence TH activity and dopamine synthesis in the rat striatum [92]. Effects of GDNF in TH activity are mediated through activation of MEK-1 and ERK [92] and this phenomenon could subserve enhanced TH + signal and improved dopaminergic functionality in ZP-grafted animals.

Hence, the neurotrophic action of GDNF and TGF-β_1 secreted by ZP cells could induce the sprouting of nigrostriatal remaining axons, leading to the host striatal reinnervation. Thus, the main advantage of ZP grafts is the long survival

of their extra-adrenal chromaffin cells, which allow them to exert a chronic dopaminotrophic action based on the delivery of GDNF and TGF-β_1, and likely other neuroprotective agents [49]. However, it should be noted that the work on this type of transplant is preliminary, and further basic studies are needed before testing its clinical applicability. For instance, since autografts are expected to be carried out in elderly population suffering from PD, more animal experimental studies on the efficacy and survival of old ZP cells after grafting are needed. Preliminary data indicate that the volume of chromaffin tissue in old ZP seems to be lower than that of young paraganaglia, with reduced trophic factor content.

Advantages of Transplanting SA Lineage Cells

The main advantage of SA cells for grafting is that, as autologous tissue, graft rejection and the need for immunosuppressant therapy are avoided, despite the fact that they do not act as dopamine secreting "minipumps." Functional effects in PD patients appear to be caused by the dopaminotrophic action in the striatum leading to improvement of dopaminergic tone and, in the case of sympathetic neurons, the capability for converting exogenous levodopa to dopamine [55–57]. Extensive striatal reinnervation characterized by abundant TH+ fibers running within the striatum has been observed after SA cell grafts when compared with PD non-grafted rats. This reinnervation arises from remaining undamaged nigrostriatal dopaminergic fibers because a recovery (even partial) of dopamine neurons of damaged substantia nigra has not been undoubtedly demonstrated with neither type of graft. Striatal reinnervation is more widespread with SA cell grafts than with transplants of fetal nigral dopamine neurons, where it is mostly dependent on the neurite outgrowth of grafted neurons and where a limited number of afferents from host neurons to transplanted cells have been detected [51, 93]. It must be considered that degeneration of the host striatonigral system continues in PD after every type of graft tested so far. Hence, striatal trophic regeneration would help reconstitute a neuronal network capable of restoring feedback-controlled release of dopamine in the nigrostriatal system. Long-term trophic action appears to be precluded by the fact that both sympathetic neurons and adrenal chromaffin cells show a very poor survival into the striatum. This is a serious disadvantage for the clinical application of this SA lineage cell substitution therapy, although extra-adrenal cromaffin cells have been observed to show better survival rate after transplantation in animal models of PD [41, 42, 72]. These types of cells represent a promising therapy based on SA lineage cells, and they can revive the use of chromaffin cells as a tool for antiparkinsonian therapy.

Carotid Body Dopamine Cells

Grafts of carotid body cells, another type of neural-crest derived cells, have proved to be effective in ameliorating functional deficits in PD rodent and primate models [7, 13, 23], and it is known that glomus cells are highly resistant

to hypoxia (carotid body cells behave as oxygen-sensing elements capable of supporting low oxygen tension) [94]. This fact would explain the high proportion of grafted glomus cells that survive long time after transplantation, as reported by the authors [13, 23]. Functional amelioration following carotid body cells grafting has been explained by the GDNF trophic action rather than by dopamine secretion [23], in the same way that the trophic effects attributed to SA cell grafts. However, the only clinical study available revealed a limited functional amelioration in PD patients grafted with autologous carotid body glomus cells, likely explained by a PD-related deterioration of old carotid body glomus cells [95]. In a randomized, double-blind and placebo-controlled study, six patients were subjected to glomus cell autotransplantation. An improvement of 26–74% at 12 months after implantation was observed in the Unified Parkinson's Disease Rating Scale III (UPDRS III) motor subscore with the patient in the "off" state. This improvement was 13–52% 1 year post-grafting. These results are remarkably similar to those obained with retinal pigmental epithelial cells, another dopaminergic cell used for transplantation. It seems that old carotid body cells degenerate within the first 3 months after grafting [23, 95], probably due to the loss of hypoxia responsivity properties of aged carotid body cells [96], along with the reduction of the volume of glomus tissue with advanced age (see Table 24.1).

Recently glial sustentacular cells of the carotid body have been demonstrated to behave as stem cells for glomus type I cells [24]. Stem cell-derived glomus cells are dopaminergic and produce glial cell line-derived neurotrophic factor, hence carotid body stem cells could be potentially useful for antiparkinsonian cell therapy. The limitation of reduced available tissue for autotransplantation in aged patients could be overcome by using this theoretically unlimited source of dopamine and GDNF-expressing glomus cells.

Fetal Mesencephalic Neurons

Transplants of embryonic dopamine neurons of the substantia nigra are the most efficient cell grafts tested so far (see Table 24.1). Fetal nigral cell grafts restore the dopamine levels in two ways: (i) releasing dopamine and (ii) inducing host striatal reinnervation through neurites arising from grafted cells, thereby leading to the restoration of the dopaminergic neurotransmission [17]. Fetal nigral cells survival rate is low, and only 3–5% of grafted neurons survive after grating [78, 79]. However, this low rate is enough to induce amelioration and tissue reinnervation even if the number of viable dopamine neurons after grafting is higher than 80,000, because the number of grafted cells has been shown to be a critical factor for the magnitude of symptomatic relief [97]. Moreover, grafted neurons that integrate with the host striatum and axodendritic synapses between host and graft have been found, suggesting that

dopamine release can be regulated, a fact that could account for good functional effects [93]. Clinical trials have shown that mesencephalic dopamine neurons obtained from human embryo cadavers survive and function after grafting in the striatum of PD patients [9, 15]. The grafts are able to normalize striatal dopamine release, and some patients have been able to withdraw from levodopa treatment for several years and resume an independent life [17, 93]. Survival of grafted mesencephalic dopamine neurons, obtained from 6- to 9-week-old aborted human embryos, has been demonstrated in the striatum of PD patients as increased [^{18}F]fluorodopa uptake, using positron emission tomography, and in histopathological studies [93, 98, 99]. Long-term survival of the dopaminergic grafts is possible at least up to 10 years post-grafting. Thus dopamine release was assessed in a patient with marked improvement of functional deficits, and binding of [^{11}C] raclopride was quantified using PET to measure dopamine D_2 receptor occupancy by endogenous dopamine. Basal dopamine release and fluorodopa uptake were normal in the grafted putamen, which probably underlies the patient's major clinical recovery [100].

Grafts of fetal mesencephalic neurons are known to restore movement-related frontal cortical activation. It is believed that impairment of the supplementary motor area (SMA) and the dorso-lateral prefrontal cortex (DLPFC) underlies akinesia in PD, a symptom that is highly refractary to pharmacological treatment. A study by Piccini et al. [101] revealed that four patients who were grafted bilaterally showed increased activation of both SMA and DLPFC at 18.3 months post-grafting. The time course of clinical improvement paralleled that of the increase of cortical activation because striatal dopaminergic neurotransmission was improved at 6.5 months with no further change thereafter. Hence it seems that improving striatal dopaminergic neurotransmission somehow restores cortical activation, leading to further clinical improvement.

Apart from ethical, practical and safety issues associated with the use of tissue derived from aborted human fetuses [17, 18], an important limiting factor for this procedure is that several embryos are needed to obtain sufficient amount of tissue because of the low survival rate of grafted neurons [9, 15, 17, 102]. Besides, since autologous tissue cannot be used as donor for transplantation, immunosuppressant therapy is needed for avoiding graft rejection. Furthermore, in the recent Denver/New York study, severe disabling dyskinesias were reported as a side effect in a significant number of PD-grafted patients [103]. This side effect seems to be caused by unbalanced partial recovery of dopamine signal in different transplanted areas of both putamina [104], and the development of dyskinesias after transplantation is not associated with excessive DA release from the grafts [105] or with excessive growth of grafted dopaminergic neurons [106]. Several authors have suggested that dyskinetic effects could be overcome by improving cell culture procedures and by carefully selecting mesencephalic dopamine cells [102]. The occurrence of grafting-induced dyskinesias or GID is a serious problem that could stop the further development of a cell therapy for PD. Accordingly, it is important that the

underlying mechanisms must be understood so that "off"-phase dyskinesias can be avoided [107].

Stem Cells

Stem cells are currently considered as the most promising future source of DA neurons for a cell-based therapy for PD [108–110]. The principal characteristics of stem cells can be summarized as follows: (i) they are undifferentiated unspecialized cells, (ii) they are able to self-renew over long periods of time, providing an almost unlimited cell source, and (iii) they can give rise to various highly specialized and mature cell types. There are two major different types of stem cells that have been studied for DA neuron generation: multipotent region-specific stem cells, isolated from embryonic/fetal or adult brain (neural stem cells, NSCs), and pluripotent embryonic stem cells (ESCs), derived from the inner cell mass of blastocysts or after nuclear reprogramming of adult somatic cells. Alternative sources of stem cells for cell-replacement therapy in PD are illustrated in Fig. 24.3.

Regardless the source of cells, the phenotype of DA neurons used for transplantation must fulfill certain criteria before they should be considered relevant to a clinical therapy. They must be capable of synthesizing and releasing DA in a controlled fashion, they need to extent axons that re-innervate the striatum and they should ameliorate motor symptoms. In summary, they must present characteristics of substantia nigra neurons [19, 110, 111] and they should be produced in large numbers.

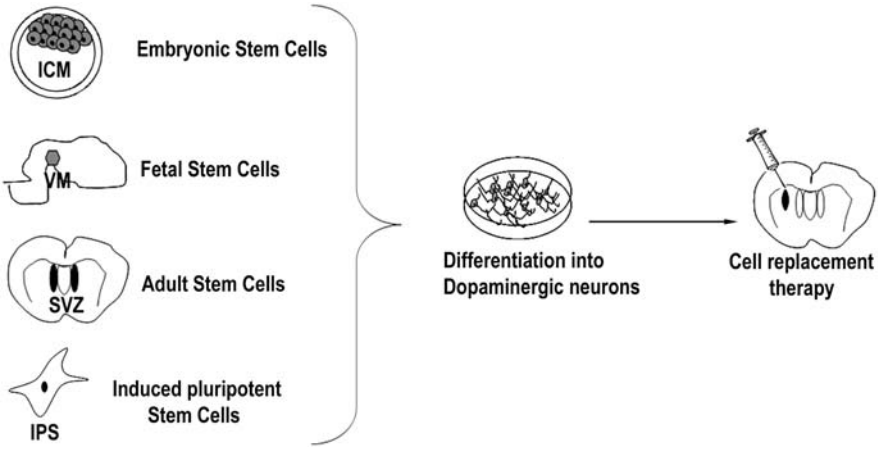

Fig. 24.3 Alternative soruces of Stem Cells for cell replacement therapy in PD. These sources include: Embryonic Stem Cells (derived from the inner cell mass (ICM) of the blastocyst); fetal Stem Cells (obtained from fetal ventral midbrain, VM); adult Stem Cells (derived from the subventricular zone, SVZ) and induced Pluripotent Stem Cells (IPS), generated after reprogramming adult somatic cells

Generation of DA Neurons from VM Neural Stem/Progenitor Cells (NSC/NP)

The earliest successful transplantation studies in rodents and human beings focused on isolating ventral mesencephalon (VM) tissue from embryos at different stages. The most promising results were obtained by grafting precursor cells isolated at the time when DA neurons began to be born. Because of the poor survival in vivo, several embryos were required for treating single parkinsonian animals and patients alike.

More recently, most of the studies have been focused on the idea of expanding VM tissue in order to reduce the number of fetuses which are required for transplantation. In one study, NSCs were isolated from VM of rat embryos and expanded using mitogens, resulting in a 10-fold increase in total number of cells and a 3-fold increase in DA neurons. Unfortunately, upon transplantation into parkinsonian animals these DA cells showed poor survival [78]. Culturing cells in semi-hypoxic conditions can also improve DA yields from VM precursors by increasing proliferation, differentiation and survival [112]. Other studies have focused on expanding and differentiating VM precursors using different cocktails such as cytokines (interleukins, LIF and GDNF) [113] or combination of several factors (BDNF, DA and forskolin) [114]. These protocols are reasonably successful in vitro, but again showed poor survival after transplantation. In the same way, a recent study has shown that treatment of mice VM neurospheres with factors involved in normal DA neuron development, such as Wnt5a, improves the differentiation and functional integration of DA neurons in vivo [115].

Generation of DA Neurons from Other NSCs

Alternatively, DA neurons can be obtained from NSCs in regions other than the VM. It has been suggested that exposure of such cells to appropriate developmental signals may induce DA differentiation. One example is Nurr1 overexpression in a NSC line isolated from postnatal mouse cerebellum in combination with VM astrocytic signals [116]. Additional studies also overexpressed Nurr1 in NSCs isolated from rat adult hippocampus and showed DA neurons upon exposure to retinoic acid or forskolin [117] or from rat adult subventricular zone (SVZ) and white matter [118]. These cells survived and differentiated into DA neurons in vivo. DA neurons have been also induced from growth factor-expanded NSCs derived from human forebrain after treatment with aFGF and different factors increasing intracellular cyclic AMP and activating protein kinase C [119], or after Bcl-XL (an antiapoptotic factor belonging to the Bcl-2 family of proteins) overexpression [120]; these cells were functional in terms of DA production and release and some of them survived after transplantation into the adult rat striatum, providing evidence

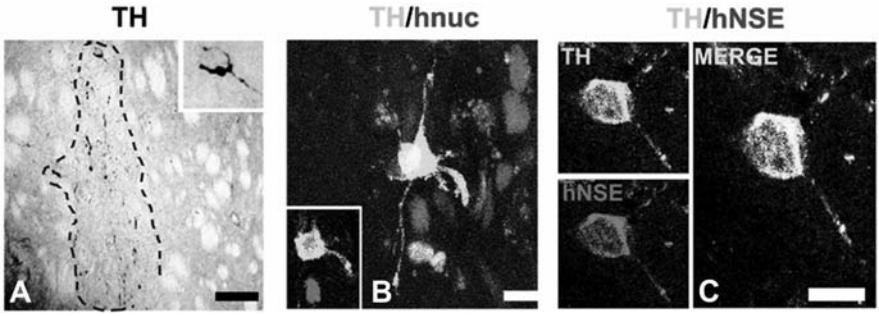

Fig. 24.4 In vivo survival of Th+ neurons derived from human neural stem cells (hNScs; hNSI line) overexpessing tha antiapoptotic gene, Bcl-XL. These Th cells were immunoreactive for human nuclei (hnuc+) and for human neural specific enolase (hNSE+). Scalar bar 100 μm in **A** and 10 μm in **B** and **C** (with permission from 120)

that they are suitable candidates for cell replacement. Figure 24.4 illustrates the in vivo survival of TH + neurons derived from human neural stem cells (hNSCs; hNS1 line) overexpressing the antiapoptotic gene Bcl-XL.

Another study has shown that overpression of Nurr1 is sufficient to drive DA differentiation of forebrain embryonic rat neural precursors in vitro [121], however, these cells are immature and not functional after transplantation. The same authors reported key factors that improve morphological and functional differentiation of Nurr1-derived DA neurons by coexpression of Nurr1, Bcl-XL and Sonic hedgehog (SHH) or Nurr1 and Mash1 [122].

Whether new dopaminergic neurons are generated from endogenous NSCs in the adult brain is controversial. Zhao et al. [123] reported continuous formation of dopaminergic neurons in the adult mouse substantia nigra, derived from precursors lining the Sylvius' aqueduct, and this generation is enhanced after lesioning the dopamine neurons of the substantia nigra. However, these results have not been confirmed by other authors that reported any evidence of neurogenesis in the substantia nigra following dopaminergic lesions [124, 125]. Finally, there is limited evidence that dopaminergic neurons can be made from NSCs in other tissues aside the brain, except for carotid body where glial sustentacular cells have been demonstrated to behave as stem cells for glomus type I cells [24]. Stem cell-derived glomus cells are dopaminergics and produce glial cell line-derived neurotrophic factor, hence carotid body stem cells could be potentially useful for antiparkinsonian cell therapy.

Generation of DA Neurons from Embryonic Stem Cells (ESCs)

ESCs offer a great potential for cell-replacement therapy as they provide an unlimited source of self-renewing cells suitable for massive expansion and directed differentiation into DA neurons for transplantation. Unfortunately

because of these properties, a significantly greater effort is required to restrict their differentiation both in vitro and in vivo, in order to achieve the desired phenotype and to avoid tumor formation.

There are two main methods to derive DA neurons from ESCs in vitro. One method consists in five-stage protocol where ESCs are initially cultured under serum conditions as embryoid bodies and then differentiated in a serum-free environment in the presence of Shh, FGF8 and FGF2 [126]. Modifications of this protocol have examined the ability to increase DA yields by providing additional developmental signals such as Nurr1 overexpression [127] or Bcl-XL overexpression [128]. DA neurons from hESCs have been generated using this method both in vitro [129] and after grafting into the rat-denervated striatum [130]; however, a mixture of midbrain and forebrain DA neurons was observed within the graft, suggesting the existence of an heterogeneous population of differentiated cells. In vitro differentiation of hESCs into dopaminergic neurons (DNs) is shown in Fig. 24.5.

The second protocol involves culturing ESC in serum-free conditions on stromal cells (such as PA6 or MS5) that have been shown to be potent inducers of neuronal differentiation. Generated neurons are subsequently differentiated, resulting in a high yield of DA neurons [131] and even greater numbers when differentiated in the presence of Shh and FGF8 [132]. Upon transplantation, these cells ameliorated rotational behavior. Similar differentiation protocols have been developed to generate DA neurons from primate [133] and human

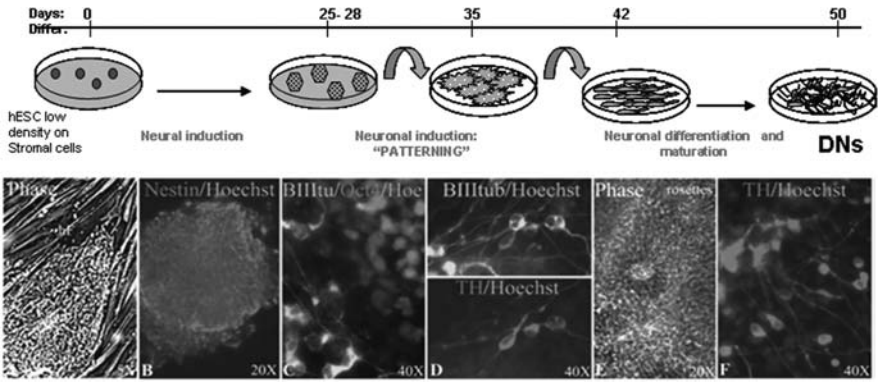

Fig. 24.5 In vitro differentiation of hESCs into Dopaminergic neurons (DNs). *Upper panel*: Schematic representation of the sequential steps designed to include a dopaminergic neuronal phenotype from hESCs. hESCs are plated at low density on stromal feeders. At the rosette stage, these neuroepithelial structures are replaced on coated dishes without feeders to continue neuronal differentiation and maturation.
Bottom panel: (**A**) Phase contrast of a colony of hESCs proliferating on human fibroblass. (**B-D**) After 12 days of differentiation, the colonies show immunoreactivity for Nestin (**B**) and some cells are already BIIItub+ and TH+, white cells in the core of the colony remain Oct4+ (**E**) Neuroepithelial structures or rosettes (**F**) Abundant numbers of TH+ cells can be observed at the end of the differentitation protocol

ESCs [134]; however, in vivo results were limited by poor survival of the grafts. More recently, two different studies have emphasized the need of glia-derived signals [135] or Noggin treatment [136] during differentiation for proper differentiation and survival of hES-derived DA neurons in vivo.

Recently, human ESC-like pluripotent cell lines (termed iPS: induced pluripotent stem cells) have been successfully established from adult human fibroblasts into which four transcription factors – Oct3/4, Sox2, Klf4 and c-Myc genes – were introduced [137]. Another group reported the generation of human iPS cells from neonate fibroblasts by a different combination of genes: Oct3/4, Sox2, Nanog and Lin28 [138]. These cells were similar to hESCs in morphology, proliferation, surface antigens, gene expression and epigenetic status of pluripotent cell-specific genes. Furthermore, these cells could differentiate into cell types of the three germ layers in vitro (including neurons expressing dopaminergic markers) [137]. Yang et al. [130] have obtained good functional results with human ESC-derived dopaminergic neurons in parkinsonian rats. However chromosomal aberrations have been observed in mid-term cultured human ESCs lines [139], a fact that can preclude their clinical application. In this context, recently Roy et al. [135] grafted dopaminergic neurons generated from human embryonic stem cells (exposed to both sonic hedgehog and fibroblast growth factor 8 with telomerase-immortalized human fetal midbrain astrocytes) and found good functional results but the grafts exhibited expanding cores of undifferentiated mitotic neuroepithelial cells, which can be tumorigenic. However, it is important to note that human NSC and precursor lines from fetal forebrain have shown genomic stability [140, 141].

The potential advantage of iPS cells, once technical limitations are solved, is obvious, not only can these cells be obtained without sacrificing embryos, and therefore without raising as many ethical concerns, but they may also represent a potential of histocompatible tissue for autologous transplantation, and it may eliminate concerns about tissue rejection that plague ESC therapy. Although further experiments are needed, stem cell therapy appears to be a good candidate for transplantation in PD, and could overcome the problem of the limited availability of human fetal neural tissue and the low survival rate of fetal dopamine neurons.

Neural Xenotransplantation

The limited availability of human fetal neural tissue poses serious limitations on clinical application of this treatment approach. Neural xenotransplanantion may circumvent many of the limitations associated with the use of human fetuses. Neural xenotransplantation can be defined as transplantation of fetal neuroblasts derived from homologous neural structures of a different mammalian species (such as pig) into the human brain. Xenotransplants of embryonic pig neural cells were shown to survive transplantation into a PD patient [142].

However, the number of surviving cells was very low suggesting that graft rejection had been taking place and there was no significant improvement of function in the patients [143]. Most probably, intracerebral xenografts require aggressive immunosuppression and, additionally, they are associated with the potential risk of animal virus transmission to humans [144].

Genetically Engineered Autologous Tissue

One of the most promising cellular substitution techniques is the possibility of engineering different cellular types before transplantation, in order to obtain cells which can release neurotransmitters or trophic factors. Besides HLA antigen could be modified in order to reduce or avoid immunological rejection. Several engineered cells have been tested in animal models of PD. Promising results have been obtained with fibroblasts engineered to secrete GDNF [28] or BDNF [30, 31], and these cells have the potential advantage that could be obtained from the patient's own skin, thereby avoiding immunological problems. Fibroblasts can be maintained in culture and engineered to produce levodopa as well, however, these types of cells have been observed to show a poor long-term integration [145–147]. Astrocytes have also been engineered to express TH and produce dopamine, and their host integration is good in animal models of PD, although their clinical efficacy has not been tested [148, 149]. These types of cells were also engineered to secrete GDNF, with good neuroprotective effects over the nigrostriatal circuit in the 6-hydroxydopamine mouse model of Parkinson's disease [29].

Retinal Pigmental Epithelial Cells

The retinal epithelium contains dopamine-producing cells, which can be maintained in culture and grafted by using microencapsulation techniques (attached to gelatine microcarriers). Experimental studies have shown that grafts of encapsulated retinal pigmental epithelial cells (RPE cells) can survive into the denervated striatum in animal models of PD, without provoking a host immune response [150–152]. PET studies with ^{18}F-dopa and ^{11}C-raclopride in MPTP-lesioned monkeys have demonstrated that these grafted cells produce dopamine and show long survival. RPE cells seem to exert a neurotrophic influence as well, and it has been proposed that transplantation of RPE cells could potentially provide a dual benefit in Parkinson's disease producing both dopamine and neurotrophic support of the basal ganglia [22]. Thus, these cells are known to release pigment-derived epithelial factor (PEDF), but according to the authors PEDF accounts for only a portion of the neurotrophic effect, indicating that differentiation increases the production of other trophic factors as well (see Table 24.1).

Transplantation of retinal pigmental epithelial cells in PD patients has been tested, and in six PD patients receiving 325,000 encapsulated retinian cells into the putamen, an average improvement of 48% at 12 months after implantation in the UPDRS motor subscore with the patient in the "off" state was observed. This improvement was sustained through 24 months post-grafting [153, 154]. No side effects were observed and, unlike grafts of fetal mesencephalic dopamine neurons, diskynesias were not reported to occurr. It seems that these cells can act as dopamine "minipumps" leading to a partial improvement of motor deficits, although their long-term integration and functionality have yet to be demonstrated. A randomized, double-blind, placebo-controlled study with human RPE cells attached to gelatin microcarriers is currently on course.

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Index