

N. Kato • M. Kawata • R.K. Pitman (Eds.)

PTSD

Brain Mechanisms and
Clinical Implications



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With 37 Figures, Including 3 in Color



Springer

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Preface

PTSD: A Window into Psychiatric Disorders

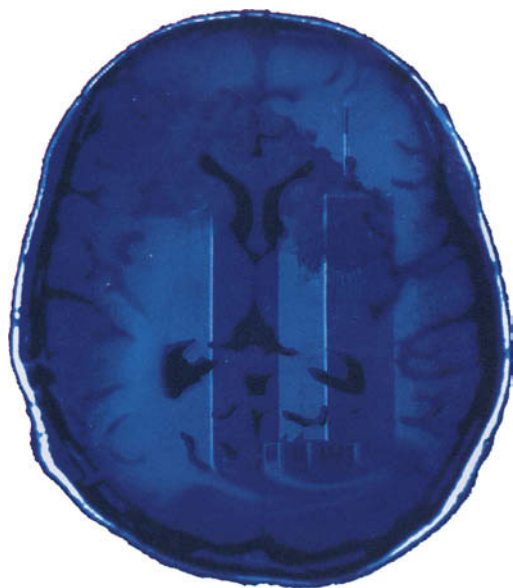
One of the bloodiest battles of the war in Vietnam was the fight for Hué City during the Tet offensive of 1968. The Viet Cong had overrun the city. American troops fought house to house and street by street to drive them out. For the men who fought there, the memories are seared indelibly like scars on the mind. Explosions and the shriek of shells rocked the city and there seemed to be snipers in every building. Each house was a mortal threat; every closed door a life and death gamble. Push it open: Would the whole room explode? Would it erupt in gunfire? Or would you risk the horror of firing into a huddle of terrified women and children? One American who lived through it remembers men beside him being shot by snipers. At least one buddy died in his arms with most of his face blown away. It was terrifying. It was absolutely exhausting and it seemed to go on forever. This was stress more fierce and agonizing than most people can even imagine.

This soldier came home months after Tet and thought it was all over, but it wasn't. He seldom found work and couldn't hold those jobs he did find. He drank a lot. He had nightmares. His whole life drifted downhill. Today he is receiving treatment and counseling at a Veterans Affairs center. That return was twenty-five years ago and the man's problems still persist. (McEwen and Schmeck: The Hostage Brain, 1994)

Posttraumatic stress disorder (PTSD) was introduced into the *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edition (DSM-III), in 1980, in response to the experiences of many patients in the United States, foremost among them Vietnam veterans. Since then the concept has been widely accepted. Nowadays this disorder is considered to be not only a sequel of disasters outside the range of usual human experience. It can also occur among ordinary people who have experienced common events such as traffic accidents, sexual assault, and child abuse. PTSD is characterized by the recurrent recall of disturbing traumatic memories, insomnia with night-

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mares, emotional numbness, and other symptoms, all of which may be long-lasting, and sometimes even of delayed onset. A frequent misconception is that trauma automatically causes PTSD. Only a minority, say 20%, of persons who are exposed to severe, even repetitive, traumatic events develop PTSD, whereas others usually recover from any acute stress reactions after a few weeks.



Eternal vigilance: Memory of September 11, 2001, invades the brain of a PTSD sufferer

Why does PTSD occur only in a subset of individuals? Reduced volume of the hippocampus has frequently been reported in patients with PTSD arising from combat trauma and prolonged childhood abuse. This provides the scientifically intriguing possibility that psychological stress may affect the volume of specific brain areas that play a pivotal role in clinical manifestations of PTSD. In primates exposed to severe social stress, prominent loss of neurons has been observed in the CA3 subfield of the hippocampus. The hippocampus is rich in steroid hormone receptors, and there is ample evidence that glucocorticoids, the adrenal hormones secreted during stress, can damage the hippocampi of experimental animals (see the chapter by Kawata in this volume). Can the hippocampal volumetric reduction seen in PTSD patients be ascribed to the atrophy induced by disastrous experience? May morphological changes also be observed in other brain regions of PTSD patients? Alternatively, does a smaller hippocampus precede the traumatic event and predispose an individual toward developing PTSD? Pitman's group (2002) addressed the chicken-versus-egg controversy regarding hippocampal atrophy. In monozygotic twins discordant for trauma exposure, they found evidence that smaller hippocampi indeed constitute a risk factor for the development of stress-related psychopathology. If a smaller hippocampus is a predisposing factor toward, rather than a consequence of, PTSD, then what is the origin of this risk factor? Is it genetic, or due to environmental circumstances during early life?

Glucocorticoids also play a role in memory consolidation, as does noradrenaline, specifically in the basolateral amygdala. These influences may help to explain the development of the strong, treatment-resistant traumatic memories found in PTSD patients. Several brain regions are suggested to participate in the processing of psychological stress, including its emotional (amygdala), contextual (hippocampus), and cognitive (prefrontal cortex) aspects (see chapters by McGaugh and de Kloet).

Accumulating evidence in PTSD points to regionally specific blood-flow patterns that suggest reduced function in the medial prefrontal and anterior cingulate cortical regions. Altered blood-flow patterns also suggest increased responsivity of the extended amygdala and insula regions (see the chapter by Liberzon). Our group (2003) recruited PTSD patients from the victims of the Tokyo subway sarin gas attack and conducted a structural MRI study with computer-assisted morphometry. The study revealed a significant volume reduction in the left anterior cingulate cortex in traumatic survivors with PTSD compared with those without PTSD, but no change at all in any other region (see the chapter by Kasai).

These and many other questions arise, and one answer introduces another chain of questions. Many are left unanswered and await further investigation. These unanswered questions stimulated a Japanese group of scientists to organize a nationwide consortium for PTSD research. Five years of work have culminated in the present book, where ideas are shared by international researchers in this fascinating field. Some of our data, for instance on neonatal isolation, indicate the development of stress vulnerability following maternal deprivation stress, as evidenced by altered gene profile and other molecular changes (see chapters by Morinobu and Honma). Even the challenge of pharmacological prevention has been covered (see the chapter by Pitman).

This volume presents a state-of-the-art overview of the basic mechanisms and clinical implications of PTSD. It is devoted to a better understanding of stress-related psychopathology, because PTSD is a window into psychiatric disorders. The aim of this volume was fulfilled by all contributors, and with the excellent assistance of staff members in my department. Finally, I wish to thank the editorial staff of Springer-Verlag Tokyo, without whose efforts this volume would have been impossible.

Nobumasa Kato
On behalf of the editors

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Part 1
Basic Mechanism of PTSD and
Stress-Related Brain Dysfunctions

Neuroanatomical and Molecular Changes in Stress Responses During Early Life: Implications for Stress Disorders

Toru Nishikawa, Akeo Kurumaji, Takashi Ito, Asami Umino, and Sumikazu Ishii

1. Introduction

Clinical observations have indicated that the symptoms and course of stress disorders including posttraumatic stress disorder (PTSD) vary with the developmental stages (Amaya-Jackson, 1995; 2000; McDermott and Palmer, 2002). For instance, childhood delayed-onset PTSD symptoms have often been described in victims of severe physical abuse, sexual abuse, or both (Amaya-Jackson 2000). These differences between the child and adult periods appear to be associated with the ontogenic development of the stress responses that compose a major defense and adaptation system to environmental stimuli. Therefore, clarifying the neuronal and molecular basis of the age-related changes in stress responsive systems should lead to a better understanding of the pathophysiology of and to a novel therapy for stress-related disorders. However, little effort has so far focused on these developmental aspects.

From this point of view, it is of interest to note that infant rats aged from about 2 to 14 days show an extremely low adrenocortical response to some systemic stressors (Fig. 1; Sapolsky and Meaney 1986; Vazquez 1998). This stress hyporesponsive period could reflect the ontogeny of the hypothalamic-pituitary-adrenocortical (HPA) axis or its regulatory neuron circuits (Sapolsky and Meaney 1986; Vazquez 1998). Postnatal development of the hippocampal glucocorticoid and mineral corticoid receptors has indeed been shown to play a substantial role in the postnatal shift in the stress-induced adrenocortical response (Vazquez 1998). It is also suggested that the alterations in the stress responses of the HPA axis could result from the maturation processes of the neuron networks and molecular cascades of a distinct stress responsive system until a certain developmental period around postnatal day 14. The matured system might contain the particular brain regions and substrates that could exhibit a developmentally regulated response to a stressor (Nishikawa et al. 1993).

To test this hypothesis, we investigated the effects of a pharmacological stressor,

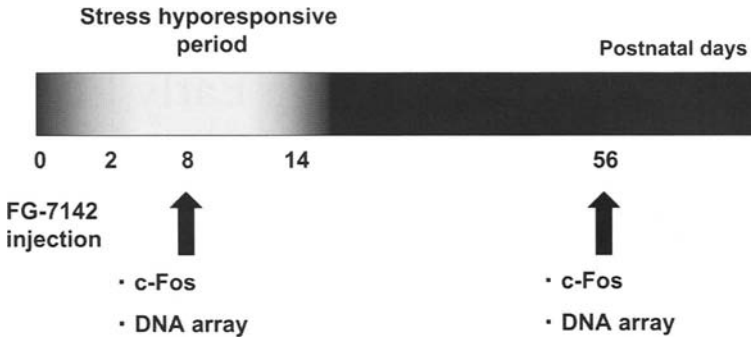


Fig. 1. Schematic representation of the postnatal development of adrenocortical responses to systemic stressors. The stress hyporesponsive period should be noted. The *arrows* indicate the postnatal days for the experiments that examined the regional and developmental differences in the gene expression following a pharmacological stressor, FG-7142. *c-Fos*, c-Fos immunostaining as a brain activity mapping; *DNA array*, exploring developmentally regulated stress-responsive transcripts using a DNA array technique

N-methyl- β -carboline-3-carboxamide (FG-7142), on the regional brain activity in infant and adult rats by monitoring the *c-fos* gene expression as the *c-Fos* immunoreactivity in brain slices (Morgan and Curren 1991). Moreover, we have explored the molecules that would respond differently to a stressor by applying a DNA array technique to the brain of the developing rats treated with an acute FG-7142 injection.

We have chosen FG-7142 as the psychological stressor, because (1) it has been widely documented that FG-7142 causes anxiety in humans and experimental animals (Adamec 2000; Sarter et al. 2001; Thiebot et al. 1988), and (2) like various types of stresses, FG-7142 has also been reported to produce the selective activation of mesocortical dopaminergic transmission (Bradberry et al. 1991; Deutch et al. 1991; Tam and Roth 1985), to increase the plasma corticosterone levels (Pellow and File 1985) and the central noradrenalin release (Nakane et al. 1994). The observations from *in vitro* studies showed that FG-7142 acts at the benzodiazepine site of the γ -amino butyric acid A (GABA_A) receptor as a partial inverse agonist (Sarter et al. 2001), and the anxiolytic and antistress property of the benzodiazepine receptor agonists (Fernandez-Teruel et al. 1991; Luddens and Korpi 1995) indicate that these behavioral and biochemical influences of FG-7142 may be associated with a reduced tone of brain GABA neurotransmission. The relevance of FG-7142 to stress disorder research seems to be further supported by recent clinical studies indicating the involvement of disturbed GABAergic transmission in the pathophysiology of PTSD (Fujita et al. 2004; Bremner et al. 2000).

2. Developmental Changes in Stress-Responsive Neuronal Systems in the Rat Brain Revealed by Brain Activity Mapping with c-Fos Immunostaining

To clarify the possible differences in the brain stress-responsive systems between the stress hypo-responsive period and adulthood, c-Fos immunostaining, as a brain activity mapping, was performed 2 h after an acute and systemic injection of FG-7142 (10, 20, and 40 mg/kg, intraperitoneally(i.p.)) or vehicle in the 8-day-old infant rats and the 56-day-old young adult rats (see Fig. 1). The rats were transcardially perfused under pentobarbital anesthesia (40 mg/kg, i.p.) with physiological saline followed by 4% paraformaldehyde (Umino et al. 1995). Immunocytochemistry was achieved on free-floating coronal sections as previously described (Umino et al. 1995). The density of nuclei stained by the anti-c-Fos antiserum in the pyriform cortex, neocortex, lateral septum, lateral habenular nucleus, paraventricular nucleus of the thalamus, retrosplenial cortex, and the central and medial nucleus of the amygdala (Swanson 1998) were quantified by counting the number of immunoreactive nuclei per unit area (Kurumaji et al. 2003).

In the adult rats, in agreement with the previous report, a sparse or marked expression of the c-Fos-like immunoreactivity was observed in the various brain areas after vehicle (Deutch et al. 1991; Umino et al. 1995; Sato et al. 1997; Singewald et al. 2003) or FG-7142 (Deutch et al. 1991; Singewald et al. 2003) injection, respectively, at the level of the rostral striatum and hippocampus (Fig. 2). The acute administration of FG-7142 (20 mg/kg, i.p.) produced a dense nuclear c-Fos-like immunoreactivity in the forebrain areas including the pyriform cortex, layers II–VI of the frontal cortex, amygdala nuclei, paraventricular nucleus of the thalamus, and anterior cingulate and retrosplenial cortex (Fig. 2). In other regions, such as the lateral habenula, and lateral septum, a moderate expression of the proto-oncogene product was detected (Fig. 2). Low or sporadic c-Fos immunostaining was seen in the hippocampus, striatum, and layer I of most of the neocortical areas. Although 40 mg/kg induced more c-Fos-like immunoreactivity than 10 and 20 mg/kg, a similar overall distribution pattern of the c-Fos-positive brain cells was observed among the three doses of FG-7142.

In the infant rats at postnatal day 8, 20 mg/kg of FG-7142 failed to cause an apparent increase in the c-Fos-like immunoreactivity in layers I–VI of the frontal, cingulate and retrosplenial cortex, lateral habenula, and medial nucleus of the amygdala, but induced a moderate expression of the gene product in the lateral septum (Fig. 2). In contrast, a similar pattern of c-Fos immunoreactivity was observed in the pyriform cortex, thalamic paraventricular nucleus, and central nucleus of the amygdala between the adult and infant rats following the FG-7142 administration (Fig. 2). In the striatum, the anxiogenic drug produced a patchy and diffuse pattern of c-Fos induction in the infant and adult period, respectively.

These findings demonstrate the regional variation in the postnatal development of the expression patterns of FG-7142-induced c-Fos in the rat forebrain. There was

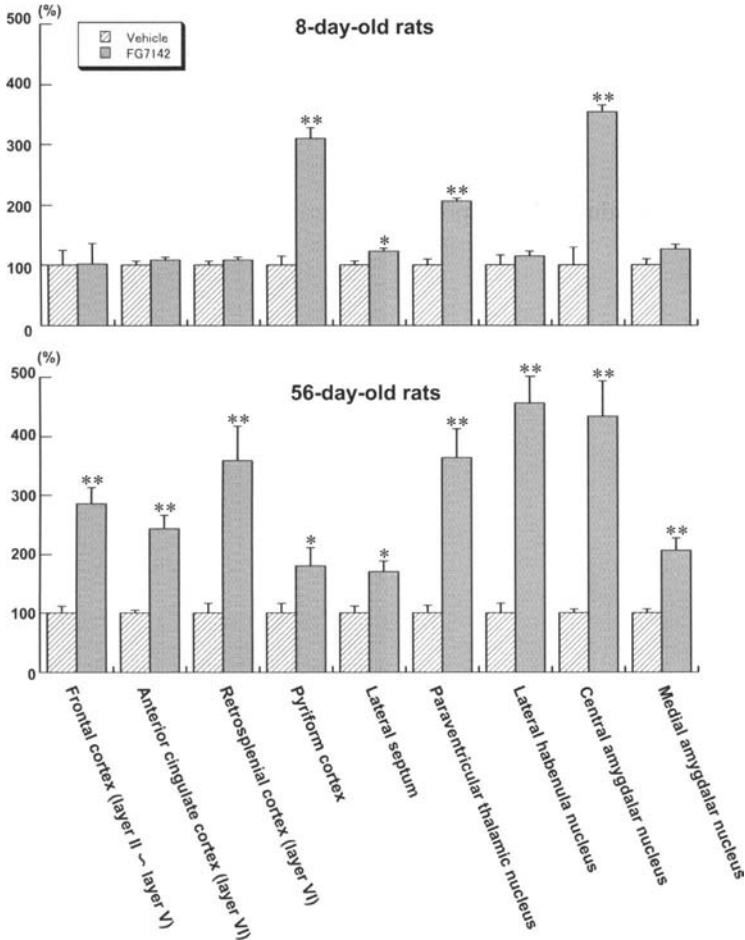


Fig. 2. The density of brain cell nuclei expressing c-Fos-like immunoreactivity in the fore-brain of rats at postnatal days 8 and 56 after the systemic administration of FG 7142 (Kurumaji et al. 2003). The rats were treated with FG 7142 (20 mg/kg, i.p.) or vehicle and were perfused 2 h thereafter. The density is expressed as the number of c-Fos-positive cell nuclei per square millimeter. Data are the mean±SEM obtained from three to eight animals and expressed as percentages of the respective control mean values which are: 8-day-old rats; frontal cortex, 18.7; anterior cingulate cortex, 92.0; retrosplenial cortex, 92.7; pyriform cortex 88.0; lateral septum, 105.3; paraventricular thalamic nucleus, 111.6; lateral habenular nucleus, 46.0; central amygdalar. nucleus, 61.3; medial amygdalar nucleus, 91.3; 56-day-old rats; frontal cortex, 84.0; anterior cingulate cortex, 83.7; retrosplenial cortex, 85.0; pyriform cortex 96.7; lateral septum, 130.6; paraventricular thalamic nucleus, 76.8; lateral habenular nucleus, 29.0; central amygdalar nucleus, 51.4; medial amygdalar nucleus, 84.2. * $P < 0.05$, ** $P < 0.01$ as compared with the retrospective vehicle-treated controls

a marked difference in the distribution and density of the FG-7142-induced nuclear c-Fos in the neocortex including the cingulate and retrosplenial cortex (mesocortex), lateral habenula, lateral septum, and medial nucleus of the amygdala between the stress hypo-responsive period and young adult period, while the c-Fos expression pattern remained rather constant in the pyriform cortex, paraventricular nucleus of the thalamus, and the central nucleus of the amygdala.

Based upon the adult c-Fos expression pattern, it can be postulated that the brain regions influenced by FG-7142 may be connected to the stress response systems. Thus, like the FG-7142 injection, systemic stresses such as noxious stimulation and hyperosmotic stress, but not processive stresses including immobilization (Senba and Ueyama 1997), induce a robust c-Fos expression in the central nucleus of the amygdala. Moreover, all four of these stressors increase the gene product in the cingulate cortex, lateral septum, medial nucleus of the amygdala, and paraventricular nucleus of the thalamus in adult rats.

The dramatic shift from the infant to adult pattern of c-Fos induction by FG-7142 could accordingly reflect the development and maturation of a stress-responsive information processing system, and be the basis for the apparent differences in behavioral modification by the anxiogenic drug between the infant and adult periods. In our experiments, the systemic injection of 20 mg/kg of FG-7142 (i.p.) caused a slight locomotor stimulation and discontinuous sniffing and rearing without overt ataxia and convulsion in the young adult rats (postnatal day 56), but exhibited sedative, hypoactive and less interactive effects on the infant rats (postnatal day 8) (Kurumaji et al. 2003). The neuroanatomical data indicating that the prefrontal cortex, lateral septum, and medial amygdala send hypothalamic efferents to the paraventricular nucleus of the hypothalamus that regulates stress responses of the HPA axis (Herman and Cullinan, 1997; Herman et al. 2002) buttress the idea that the much lower c-Fos induction by FG-7142 in these origin areas of hypothalamic projections in the infant rats as compared with the adult rats would, at least in part, be associated with the infant hypo-responsiveness of the HPA axis to stress. In terms of the ontogenic changes in the lateral habenular c-Fos expression after the anxiogenic injection, it should be noted that interruption of the habenular efferent pathway at the level of the fasciculus retroflexus at either 3 or 70 days of age affected the stress-related anxiety (elevated plus-maze test) and activity levels (locomotion and grooming in open-field test) in different ways (Murphy et al. 1996). This experiment suggests that the habenula might play distinct roles in modification of the stress response system as a function of age (Murphy et al. 1996).

3. Exploration of Developmentally Regulated Stress-Responsive Transcripts in the Rat Neocortex

The present brain activity mapping data indicate the presence of the mature or adult-type information processing system specifically related to a type of stress including

FG-7142. This system may, at least, implicate the neocortex, because the brain region exhibits the most prominent alterations in the c-Fos induction pattern between the infant and adult periods. It is also postulated that the molecular cascade of the matured system may contain the genes that show a stress-induced change in their neocortical expression during the adulthood, but not during the infant period. Therefore, we have performed in the neocortex of the male C57BL mice, a DNA microarray technique [the Mouse cDNA microarray (Agilent)] to isolate the candidate genes that are differentially expressed between postnatal days 8 and 56 at 1 h after the systemic administration of FG-7142 (20 mg/kg, i.p.) (Kurumaji et al. 2005).

Equal amounts of total RNA prepared from the neocortex (the dorsal part of the cerebral cortex divided along the rhinal fissure) of the individual rats of every experimental group using the Quiagen RNeasy Midi System (Quiagen, Valencia, CA, USA) were pooled. Twenty micrograms of the pooled total RNA was reverse transcribed using the oligo dT12-18 primer and aminoallyl-dUTP. The synthesized cDNA was labeled by reaction with dye, NHS-ester Cy3 (the vehicle-treated sample) or NHS-ester Cy5 (the FG 7142-treated sample) (Hughes et al. 2001). The labeled cDNA was applied to the DNA microarray (Mouse cDNA Microarray, Agilent). After washing, the microarray was scanned on a microarray scanner (ScanArray 5000, GSI Lumonics) and the image was analyzed using software (QuantArray, GSI Lumonics). The signal intensity of each spot was calibrated by subtraction of the intensity of the negative control, and was normalized to the global value of all the genes provided on the membranes. The expression of a gene on a specific spot was considered as relevant if the signal intensity was greater than twice the SD of the background.

In the DNA microarray experiments, we found eight developmentally regulated FG-7142-responsive genes, designated as axg 6~13 (anxiogenic responsive transcript 6~13), that met the following criteria for the candidate molecules associated with a matured stress system: (1) the signal intensity of spot labeled by Cy3 (vehicle-treated sample) was more than 500 and the calculated expression ratio of Cy5/Cy3 was greater than two or less than 0.5 in the adult animals, and (2) there were no differences in the hybridization signals between the vehicle-treated controls and the FG-7142-injected animals. No down-regulated genes by FC-7142, which exhibited a spot signal intensity less than 0.5, were observed in the adult neocortex. Further studies by the quantitative real-time RT-PCR method using the LightCycler system (Roche, Penzberg, Germany) revealed that FG 7142 induced a statistically significant increase in the rates of the mRNA levels of each of these eight genes to those of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Robbins and McKinney 1992), in the mouse neocortex on postnatal day 56, but not day 8. The neocortical expression of axg6, axg7, and axg8 were also upregulated by immobilization stress and another anxiogenic factor, yohimbine, in the 56-day-old mice.

The differential regulations of these eight transcripts by the anxiogenic drug between the stress hypo-responsive period and maturity suggest that mammalian brains might process the information from the stress stimuli by the distinct sets of molecules between the two stages of postnatal development. Moreover, the eight anxio-

genic-responsive transcripts could be novel members of the molecular cascades involved in the stress responses, because none of these transcripts have so far been reported to be responsive to any kind of stress.

4. Clinical Implications

Our studies presented here demonstrate the marked neuroatomical and molecular changes in stress responses during early life. The developmental changes suggest that stressor-specific neuron circuits and signaling pathways may mature at the possible critical period between the stress hyporesponsive period and the adult period in mammalian brains and the acute and long-term effects of stresses on behavior should change across the critical period. It is proposed that a similar developmental mechanism could underlie the age-related transfigurations of the psychiatric symptoms and their courses of PTSD and other stress disorders in humans. This view also suggests the necessity of the development-based therapy for these mental dysfunctions. Therefore, the developmentally regulated FG-7142-responsive genes and/or their protein products would be implicated in the pathophysiology of a group of stress disorders and be suitable targets for the development of a novel and age-directed treatment or prophylaxis for these illnesses.

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Cortisol and PTSD: Animal Experiments and Clinical Perspectives

E. Ronald de Kloet and Melly S. Oitzl

1. Introduction

A fundamental issue in the psychobiology of a traumatic experience is how circulating stress hormones are implicated in the pathogenesis of posttraumatic stress disorder (PTSD) (Pitman 1989; Gilbertson 2002; Ohtani et al. 2004). This issue is important because the onset and progression of the disorder appears to be facilitated by a low circulating cortisol tone (Yehuda 2002). Hence the hypothesis was proposed that the “development of PTSD is facilitated by a failure to contain the biological stress response at the time of the trauma. As a result a cascade of central stress reactions may lead to intrusive recollections of the event, avoidance of reminders of the event, and symptoms of hyperarousal,” (Yehuda 2002). Indeed corticotropin-releasing hormone (CRH) levels in the cerebrospinal fluid are high in the face of low circulating cortisol concentrations. This suggests that inadequate cortisol feedback in the brain may be implicated in PTSD.

In this chapter, we address the question why cortisol action is inadequate to contain central stress reactions to traumatic events. The action exerted by cortisol in the brain is mediated by high affinity mineralocorticoid receptor (MR) and the lower affinity glucocorticoid receptor (GR) (de Kloet et al. 1998). The two receptor systems operate in neural networks as a binary control mechanism in the regulation of signaling cascades underlying distinct domains of emotional and cognitive processes (Oitzl and de Kloet 1992; de Kloet et al. 1999). By operating as a dual control mechanism, MR and GR are considered to be an interface between genetic and traumatic inputs that shape the organism's ability to cope with stressors. It is conceivable, therefore, that an imbalance in MR-mediated and GR-mediated actions is a significant factor in the development of a phenotype vulnerable to the precipitation of PTSD. A prominent example of such a vulnerable phenotype is the newborn infant, as will be illustrated for the rat and mouse.

2. Glucocorticoid Function in Control of Stress Reactions

Glucocorticoid secretion from the adrenals is enhanced at the beginning of the active period during the circadian rhythm and in response to stressors. This enhanced glucocorticoid secretion is due to increased activity of the hypothalamic-pituitary-adrenal (HPA) axis after activation of the CRH/vasopressin (AVP) producing parvocellular neurons of the paraventricular nucleus (PVN) and the release of adrenocorticotrophic hormone (ACTH) from a proopiomelanocortin (POMC) precursor in the anterior pituitary. The stimulation of the HPA axis can be *direct* by ascending monosynaptic projections from the brain stem to the PVN conveying sensory information as is the case for pain, heat, cold, noise, inflammatory, and immune stimuli. The HPA axis also can be activated *indirectly* through processing of information that is associated with the stressor and in this way even purely psychological processes are powerful stressors. For processing of psychological information networks in limbic forebrain regions such as the hippocampus, amygdala, and frontal cortex are essential. Today's wisdom ascribes to these regions an emotional (amygdala), a contextual (hippocampus), and cognitive (prefrontal cortex) content to stressful inputs. These functions play a role in appraisal of real or imagined situations for their destabilizing potential. The outcome of the appraisal process is then conveyed transsynaptically to the hypothalamic CRH neurons (Herman et al. 2003).

CRH organizes through CRH-1 receptors not only the neuroendocrine, but also the sympathetic and behavioral "fight-flight" response to a stressor. Recently, urocortins II and III acting via the CRH-2 receptor system were discovered (Heinrichs and Koob 2004; Hsu and Hsueh 2001; Reyes et al. 2001). The CRH-1 and CRH-2 receptor systems have a partly overlapping central nervous system (CNS) distribution in the hypothalamus, brain stem, and limbic system that matches their CRH and urocortin terminal fields. Generally, CRH administered icv mimics the initial stress response and produces anxiogenesis, while urocortins have anxiolytic properties. Although not yet firmly established, it seems that the CRH-CRH-1 receptor and the urocortin-CRH-2 receptor networks represent acute and late recovery components of the stress system, respectively, that interact in balance, along similar criteria as the sympathetic and parasympathetic nervous system, and the proinflammatory vs anti-inflammatory Th1/Th2 balance in the immune system.

Adrenal glucocorticoids (cortisol in man, corticosterone in rodents) operate in the acute and recovery phase. Their role in initial stress reactions became apparent from corticosterone actions in rapid agonistic behavioral responses (Kruk et al. 2004) that can be blocked by mineralocorticoid antagonists (Haller et al. 2000). Also recent electrophysiological studies on the mechanism showed rapid nongenomic actions in glutamate transmission (Karst et al. 2003). In the limbic structures such rapid actions seem implicated in the appraisal phase of novel, confronting situations and in the organization of an appropriate behavioral response. Much better investigated are the slow actions of glucocorticoids that act oppositely and block the initial stress reactions. It is now common knowledge that glucocorticoids dampen the initial stress reaction, to prevent them from overreacting (Munck et al. 1984).

Indeed Marius Tausk (1952) commented: "Cortisone treatment is appropriate where the defensive reactions of the organism cause more damage than the agent to which they defend, or as metaphor: glucocorticoids protect against the water damage caused by the fire brigade." In the slow mode of action the hormones are part of the recovery phase preparing the organism for future events. It is this latter action that provided the glucocorticoids with the power of a miracle drug in its cure of inflammatory and immune disorders. Glucocorticoids are key mediators in glucose homeostasis from appetite and choice of macronutrients with energy need, mobilization, and disposition (Allaman et al. 2004; Peters et al. 2004). Glucocorticoids, in concert with a host of transmitter and peptide mediators, thus coordinate the body and brain mechanisms underlying coping with stress and energy metabolism.

3. Binary Receptor System

Under basal conditions, only the high-affinity receptor, MR, is activated, while both receptors are occupied at times that steroid levels are high, i.e., after stress (Reul and de Kloet 1985). The stress receptor, GR, is expressed in every cell, but unevenly, with highest concentrations in the PVN, corticotrophs, cortical, and limbic neurons as well as in the ascending serotonergic and catecholaminergic neurons. MR occurs only in high amounts in limbic neurons where they are colocalized with GR (van Steensel et al. 1996; Han et al. 2005; Nishi et al. 2004). The best investigated actions mediated by MR and GR are genomic. Using gene expression profiling technology, partially overlapping responsive gene patterns have been identified in the hippocampus (Datson et al. 2001). Yet, current profiling technology allows detection of only medium to high abundant genes in laser-dissected cell fields (Datson et al. 2004). Electrophysiology combined with single cell profiling from individual neurons has established a number of criteria for genomic glucocorticoid actions (Nair et al. 2004).

First, glucocorticoid actions are conditional, i.e., their action depends on the nature and context of the stressful stimulation; they have therefore an enormous diversity in actions. Yet, certain generalizations can be made. Genomic actions mediated by GR usually suppress transiently raised excitatory stimulation by stressors. This is exemplified by electrophysiological studies, which show that glucocorticoids attenuate the norepinephrine-induced blockade of action potential accommodation in hippocampal CA1 neurons (Joëls and de Kloet 1989). Those mediated by MR usually have opposite effects and enhance excitatory transmission (Joëls and de Kloet 1992).

Second, the genomic steroid actions are slow in onset and long lasting. However, much has been learned in recent years about the selectivity of signaling pathways. Thus, GRs not only exert transactivation through interaction with glucocorticoid responsive elements (GRE) on the DNA, but also more rapid transrepression through blockade of membrane driven transcription factors by GR monomers (Meijer et al. 2000). Likewise, patterns of coactivator and corepressor molecules can

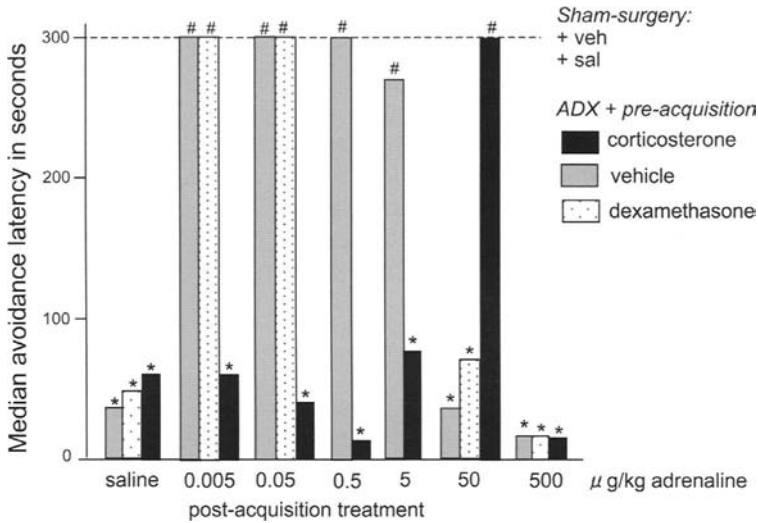


Fig. 1. Retention of step-through inhibitory avoidance response. Corticosterone desensitizes the effect of adrenaline up to ten thousand-fold, dexamethasone is ineffective. Forty-eight hours after adrenalectomy (ADX), rats were treated with corticosterone or dexamethasone (300 µg/kg s.c.) 60 min prior to the acquisition session followed by an adrenaline injection (doses of 0.005 to 500 µg/kg s.c.) immediately after the acquisition trial. Retention was tested 24 h later. Hash, $P < 0.05$ vs ADX veh+sal; asterisk, $P < 0.05$ vs Sham+ veh+sal. Adapted from Borrell et al. (1984)

modulate in receptor-selective fashion gene transcription (Meijer et al. 2005).

Third, the use of steroids for brain studies has a caveat, because synthetic high affinity steroid ligands are also good substrates for multidrug resistance P glycoprotein (mdr Pgp), which hampers their penetration of the blood–brain barrier. Dexamethasone, for instance, penetrates the blood–brain barrier poorly for that reason and therefore primarily targets the pituitary in suppression of stress-induced HPA activation (de Kloet et al. 1974, 1975; Meijer et al. 1998).

By taking the data of our research together (de Kloet et al. 1998, 2005) we have postulated that the balance of MR-mediated and GR-mediated actions is critical for homeostasis and health. MR controls the sensitivity or threshold of initial stress reactions that facilitate in a *proactive* mode the immediate coping of the organism with novelty or challenge. GR, in *reactive* mode, facilitates recovery and promotes the storage of information relevant for coping with future events. Both receptor types mediate, therefore, glucocorticoid actions that are sometimes opposite or other times overlapping and synergistic, in different domains of the organisms coping with a stressor.

4. Behavior

Glucocorticoids thus change excitability of cells and circuits that underlie emotional and cognitive processing of novel information. The nature of the behavioral responses is, however, determined by the context and the type of receptor involved (de Kloet et al. 1999). In our studies in rats and (mutant) mice, posttraining GR activation promotes the consolidation of new information. MR is not active in information storage, but rather is implicated in the appraisal process and the choice of an appropriate behavioral response to deal with the situation (Oitzl and de Kloet 1992; Oitzl et al. 2001). This conditional effect exerted by the steroids may explain why memories will be longer lasting when a situation is particularly arousing (Sandi 2004). It was demonstrated that corticosteroid effects on consolidation of memory in fear conditioning, Morris water maze, and object recognition depend on emotional arousal as tested pharmacologically by manipulating concomitantly catecholaminergic input into limbic structures (McGaugh 2004; Roozendaal et al. 2004). In this respect, it has been known for many years that glucocorticoid replacement of adrenalectomized (ADX) rats attenuates the facilitatory effect of epinephrine on the retention of an inhibitory avoidance response (Fig. 1) (Borrell et al. 1984). In the presence of glucocorticoids, the dose of epinephrine administered posttraining needed to be increased ten thousandfold in order to enhance retention of behavior in this fear-conditioning test.

Interactions between the (basolateral) amygdaloid nucleus, the hippocampus, and the frontal cortex are crucial in this respect (McGaugh 2004; Adamec et al. 2004). Corticosterone and acute stress are claimed to impair retrieval of information if administered prior to testing. On the other hand, corticosterone facilitates learning of novel associations (Beylin and Shors 2003), and eliminates behavior that is of no more relevance (de Wied 1997). A special feature of this had been already demonstrated more than two decades ago in a forced extinction paradigm. It was found that the extinction of the fear-related response depended selectively on the naturally occurring glucocorticoid corticosterone, because steroids such as dexamethasone and deoxycorticosterone were not effective (Bohus and de Kloet 1981) (see Fig. 2). Rather than impairing a retrieval process, corticosterone allows the acquisition of new, more appropriate contextual information. This is an acceptable alternative interpretation of the data obtained with preretrieval administration of glucocorticoids, and which is also in line with the current view on extinction as an inhibitory form of learning in contrast to the excitatory form of conditioning (Myers and Davis 2002; Maren and Quirk 2004).

5. Implications for PTSD

The knowledge of glucocorticoid physiology with its ramifications in behavior has important implications for understanding the pathogenesis of PTSD. Recent reports

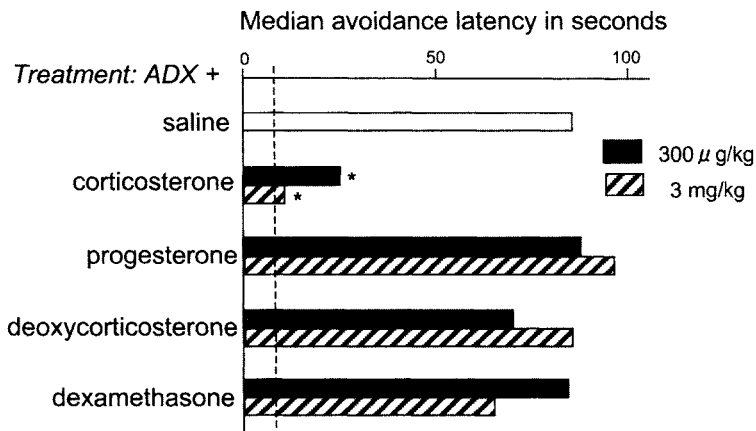


Fig. 2. Corticosterone normalizes extinction behavior. Rats were adrenalectomized and treated with various steroids (300 µg/kg and 3 mg/kg s.c.) 60 min before forced extinction. On the following day, retention of inhibitory avoidance behavior was measured. Forced extinction (exposing the rats for 5 min to a compartment where they had received a footshock 24 h earlier) eliminates inhibitory avoidance in sham-operated rats (*dotted line*) and rats treated with corticosterone. The other steroids were ineffective. Asterisk, $P < 0.05$ vs saline-treated ADX. Adapted from Bohus and de Kloet (1981)

suggest that in PTSD patients, cortisol exerts an excessively *strong* glucocorticoid feedback signal on pituitary ACTH release, which results in downregulation of HPA axis activity (Yehuda 2002). As such, this potent pituitary feedback explains the low cortisol tone. In support of this phenotype, dexamethasone shows an enhanced efficacy in suppression of ACTH and cortisol levels (Duval et al 2004; Yehuda et al. 2004). However, pituitary-adrenal response in the dexamethasone/CRH test is facilitated (Rinne et al. 2002). Moreover, levels of CRH in the CSF are elevated, while heart rate and other parameters suggest an increased sympathetic tone. Hence, a dissociation of central and peripheral activation of the HPA axis is now obvious; high central stress system activity occurs in the face of low circulating cortisol concentrations.

The lower cortisol tone leaves circuits underlying fear and other emotions underexposed to the hormone. As a consequence increased MR over GR activity can be predicted. It would be of interest to examine if this phenotype, characterized by increased MR-mediated over GR-mediated actions, would allow the excessive “out-of-context” appearance of fear responses and memories. If this were the case, it would call for studies where cortisol is administered in attempts to facilitate extinction of the traumatic memory. Indeed, in two recent, rather preliminary studies, supporting evidence was found. A low dose of cortisol administered daily for 1 month alleviated cardinal symptoms of PTSD (Aerni et al. 2004). The second study as-

sumed a function of cortisol in extinction of fear-motivated responses. Alternatively, the same researchers reported that high doses of cortisol in patients undergoing cardiac surgery are associated with a lower intensity of chronic stress and PTSD symptoms at 6 months after surgery, showing the importance of glucocorticoid actions during acquisition of traumatic memories (Schelling et al. 2004). In another study, the synthetic glucocorticoid dexamethasone did not further impair the declarative memory in PTSD patients, while it clearly did so in control subjects (Bremner et al. 2004). As pointed out above, low dexamethasone in fact depletes the brain of endogenous cortisol.

While the dispute about whether the behavioral effects represent either cortisol-impaired retrieval or cortisol-facilitated extinction might appear semantic at first glance, it appears crucial for understanding the therapeutic efficacy of cortisol in the treatment of PTSD. The interpretation in terms of a cortisol-impaired retrieval process implies a decay of fear memory in the presence of cortisol, which has not been proven yet. In contrast, an extinction process facilitated by cortisol extends to a mechanism that allows the modification of fixed response patterns, and, thus, to actually extinguish inappropriate fear-related behaviors.

6. Perinatal Life Presents a Vulnerable Phenotype

Newborn rodents experience a so-called stress hyporesponsive period (SHRP), which implies that mild common stressors are unable to trigger an ACTH or corticosterone response from postnatal day 4 to postnatal day 14 in the rat, while the brain's stress circuitry markers like CRH are very responsive (Levine et al. 2000). For instance, exposure to a mild stressor, e.g., novelty or an intraperitoneal saline injection, hardly produces a response in the immature animal, while the same procedure triggers a profound response in the adult. The pituitary and adrenal are hyporesponsive, but mild stressors trigger in the pup a profound CRH mRNA response, while in the adult it takes about 4 h. This might not at all be exclusive for rats, because an SHRP has recently been proposed in children (Gunnar and Donzella 2002). The condition of quiescent peripheral stress responsiveness and highly active central stress system activity is reminiscent of a clinically relevant vulnerable phenotype for PTSD.

The most powerful effect of disruption of the SHRP is achieved when the pup is deprived of the dam's care, i.e., feeding, licking, and grooming. The separation of mother and pup for 24 h not only activates the HPA axis to a higher set point, it also sensitizes the axis to the very same stressors that did not evoke a corticosterone response in the well-groomed infant. After maternal deprivation, mild stressors are capable of triggering an HPA response. Anogenital stroking of the pup with a warm wet artist brush every 8 h for 45 s (which forces the pups to urinate) reinstates quiescence again on the level of pituitary ACTH release and also normalizes the exaggerated stress-induced hypothalamic CRH and *c-fos* responses. Additional feeding also normalizes adrenal sensitivity and circulating corticosterone back to SHRP sta-

tus (van Oers et al 1998b, 1999). The data suggest that metabolic signals as well as sensory signals are important for maintenance of the SHRP.

The underlying mechanism to maintain the SHRP appears to be a potent glucocorticoid feedback signal at the level of the pituitary. This is concluded from studies showing that systemic (Schmidt et al. 2005), rather than central glucocorticoid antagonist application (Yi et al. 1993) disrupts the SHRP. The GR antagonist produces a profound rise in pituitary POMC mRNA expression and in circulating ACTH and corticosterone levels. In contrast, CRH mRNA expression was downregulated after the systemic GR antagonist, further reinforcing a pituitary glucocorticoid site of feedback action in the maintenance of the SHRP. Maternal deprivation increases responsiveness of the neural stress circuitry; MR and GR mRNA expression in hippocampus, as well as GR mRNA in PVN are downregulated. In contrast, hippocampal GR is upregulated irreversibly under conditions of intensified maternal care. This stable upregulation is due to demethylation of a cysteine residue at the 5' NGF1-A binding region in the exon 1-7 promoter (Weaver et al. 2004).

In light of the strong corticosterone response to maternal deprivation, it seems logical that in the literature this hormone is held responsible for the outcome of mother-pup separations. Nevertheless, caution should be taken in the interpretation of these data, as firm proof for the causality of corticosterone is lacking. For instance, pretreatment of pups with dexamethasone completely abolished the corticosterone response to maternal deprivation, but did not affect the central effects of mother-pup separation (van Oers et al. 1998b, 1999).

7. Long-Term Outcome of Maternal Deprivation: Some Get Better and Others Get Worse

Half a century ago Seymour Levine demonstrated that handling of rat pups (removing the animals daily for 15 min) during their postnatal development produced a lasting suppression of emotional and neuroendocrine reactivity (Levine 1957). Further in-depth studies (Meaney et al. 1988; Liu et al. 1997, 2000) extended these findings to the model of low and high grooming mothers, underscoring that maternal care matters and that alterations of maternal care during development affect the function of the individual during adulthood.

It seems logical that if increased maternal care is beneficial for the development of the infants, then a prolonged maternal absence or neglect is unfavourable or even harmful. One of the best studied models employs a separation paradigm of 3 h daily throughout the SHRP (Plotsky and Meaney 1993). The repeated separations produced a phenotype in later life characterized by enhanced emotional and HPA responses to stress as well as elevated hypothalamic and amygdaloid CRH mRNA expression. MR expression was enhanced in hippocampus, but GR was not affected. Accordingly, the repeated separations do produce lasting changes in the stress system, but only partly of the PTSD phenotype. However, the repeated separation para-

digim is based on the assumption that the induced effects are cumulative and unidirectional. However, other studies showed that the timing of maternal deprivation proved to be crucial. van Oers et al. (1998a) showed that maternal separation for 24 h at the beginning or toward the end of the SHRP resulted in either an increased ACTH or a decreased ACTH response to stress in the young 21-day-old animal. Differences based on sex were also found. Maternal deprivation for 24 h at the beginning of the SHRP downregulated hippocampal GR in adult male rats, which was further enhanced if the adrenals were stimulated with ACTH at the time of maternal deprivation. In contrast, in females deprived as pups, GR was increased at adulthood and this increase was further enhanced upon neonatal ACTH injection (Suntanto et al. 1996). Downregulation of MR was only observed in the deprived males. While virtually all rats and mice subjected for the first time to maternal deprivation of more than 4 h will react with an immediate activation of the HPA axis (Schmidt et al. 2004), the long-term consequences of maternal deprivation are much more subtle. Apart from the duration of the separation, they depend on the time point of the separation during the SHRP, gender, as well as the genetic background (van Oers et al. 1998a).

In a large study using one cohort of Brown Norway rats that were subjected at postnatal day 3 to 24 h of maternal deprivation, we examined, in a longitudinal as well as a transversal study design, stress system activity and cognitive performance in relation to an index for brain plasticity (Oitzl et al. 2000). This study revealed that exposure to a traumatic experience during the neonatal period increased the number of animals that showed either bad or good cognitive performance when compared with controls. The number of good performers increased at senescence twofold, and that of bad performers with a factor 1.5. Aging of the control animals provided a large group of partially impaired animals (45% of the rats), while from the maternally deprived animals only a few (12% of the rats) were partially impaired. Thus, maternal deprivation leads to either successful aging or senility at the expense of the population of partially impaired animals. We also found that cognitive performance correlated with the expression of brain-derived neurotrophic factor (BDNF) in the hippocampus: the better the animals learned, the higher was the expression of BDNF mRNA in the hippocampus (Schaaf et al. 2001).

What is the role of the stress system and of genetic background in this dichotomization of cognitive performance at senescence as a result of maternal deprivation? In the same study, parameters of HPA activity were measured. If exposed to novelty the response of corticosterone slowly attenuated during the aging process and was lowest at senescence, while ACTH increased. In maternally deprived rats, peak levels of stress-induced corticosterone were, at midlife, far higher than in the controls, but much lower at young age. At senescence, particularly after exposure to more severe stressors, the corticosterone response was less than observed in the controls (Workel et al. 2001), thus, possibly resembling a PTSD phenotype. It would be of interest, therefore, to examine whether the extent of midlife stress is a determinant in selecting a trajectory toward either successful aging or senility, and, if so, which gene patterns are being activated under such conditions.

8. Conclusion

A frequent misconception is the assumption that trauma automatically causes PTSD. Only a minority, about 10%–40%, of persons who are exposed to one severe or to repetitive traumatic events develops PTSD, while others might develop (comorbid) depression (Davidson et al. 2004). The outcome of the “early handling” studies in animals seems to suggest that all individuals are affected in later life in the same mode and direction (Meaney et al. 1988; Liu et al. 1997, 2000; Plotsky and Meaney 1993). Other studies (Oitzl et al. 2000), however, clearly demonstrate that the maternal deprivation paradigm amplifies (genetically determined?) individual differences (some rats gain from a traumatic experience, other lose the ability to cope), much like the way in which 10%–40% of the individuals experiencing trauma develop PTSD. Thus, factors other than the traumatic experience are important determinants for interindividual variations (van der Hart et al. 2004).

PTSD is a disorder with a defined onset and progression, but, if not every individual is affected, what is determining the interindividual differences, even if a vulnerable phenotype is exposed to trauma? A low cortisol level might therefore be a permissive, but not a necessary condition, and other factors are involved. Genetic predisposition is considered such a factor that may generate a vulnerable phenotype that is sensitive to adverse childhood experiences as a risk factor for the precipitation of PTSD or depression during adulthood (Bremner et al. 1993; Carlson et al. 2001). Examples are the presence of the short vs the long allele in the 5-HT transporter (Caspi et al. 2003) and GR polymorphisms (van Rossum and Lamberts 2004).

However, chronic childhood adversity appears to render HPA axis function permanently hyperresponsive rather than hyporesponsive, resulting in a strongly increased ACTH and cortisol output to a combined dexamethason/CRH challenge test, as well as to a psychological stress challenge in female victims of sustained childhood abuse (Rinne et al. 2002). These effects are due to an increased responsivity of the hypothalamic CRH/AVP drive and turned out to be independent of a concurrent PTSD. A concurrent PTSD mitigates the net ACTH and cortisol output in chronically abused and nonabused subjects (Heim et al. 2001). This finding hints at two different and independent pathophysiologic mechanisms underlying the neuroendocrine sequelae of chronic childhood abuse and PTSD. Chronic childhood abuse is likely to be correlated with an increased CRH/AVP drive and PTSD appears to be associated with an increased glucocorticoid feedback inhibition and low cortisol levels (Heim et al. 2002; Newport et al. 2004). If individuals with increased pituitary glucocorticoid feedback inhibition and central glucocorticoid resistance are at risk of PTSD, this would call for studies on possible associations with genetic polymorphisms in corticosteroid signaling.

Much progress has been made in recent years in describing a potential vulnerable phenotype for PTSD. Hallmarks are the low circulating cortisol levels, the strong pituitary feedback, and inadequate containment of central stress reactions observed in patients (Yehuda 2002). Similar features are presented in certain animal models, of which the neonatal rat (Levine et al. 2000), or even the fetus (Seckl 2004), are the

most prominent vulnerable phenotypes for traumatic experiences. Progress can be expected by further in-depth analysis of the executive part of the stress system, such as the frequency and amplitude of HPA pulsatility (Young et al. 2004), and in understanding of the receptive part by measuring gene variants and polymorphisms in the corticosteroid receptor types. It can be expected that the “balance theory” of the two opposing stress system modes, represented among others by MR-mediated vs GR-mediated actions underlying homeostatic control and behavioral adaptation, will be put to test to further understand the onset and progression of stress-related disorders such as PTSD and depression. It will be a tall order to sort these things out.

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Stress and Corticosteroid Receptors

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1. Introduction

The hypothalamo-pituitary-adrenal (HPA) axis is the main hormonal system involved in stress response. Neurons in the hypothalamic paraventricular nucleus secrete adrenocorticotrophic hormone-releasing hormone (CRF) and vasopressin, which then synergistically induce the secretion of adrenocorticotrophic hormone (ACTH) from corticotrophs in the anterior pituitary, activating the secretion of the glucocorticoids from the adrenal cortex. Glucocorticoids (cortisol in human and corticosterone in rodents) readily enter the brain from the circulating blood and bind to their receptors to induce the secretion of CRF and ACTH for feedback regulation to keep homeostasis through the HPA axis. Over the past decades there has been increasing evidence supporting a critical role for the hippocampus in stress response (McEwen 1999). Neurons in the hippocampus express receptors for circulating glucocorticoids, and animal models of repeated stress caused dendritic atrophy in CA3 pyramidal neurons (Woolley et al. 1990; Watanabe et al. 1992; Magarinos and McEwen 1995; Sousa et al. 2000). In the primate prominent pyramidal neuron examples of damage such as dendritic atrophy and pyknotic changes were observed in the CA3 area of animals subjected to repeated social stress and long-term treatment of cortisol (Uno et al. 1989, Sapolsky et al. 1990). These results suggest that dendritic atrophy of the pyramidal neurons may be one of the factors contributing to the clinically observed decrease of hippocampal volume in humans suffering from stress-related disorders including the posttraumatic stress disorder (PTSD) (Bremner et al. 1995). Transsynaptic inhibitory projection of the hippocampus to hypothalamic paraventricular nucleus has been demonstrated. Therefore, the hippocampus has been the focus of the stress response not only due to its susceptibility to stress-related damages but also to its negative feedback regulation of the stress response via the HPA axis (Herman and Cullinan 1997). In contrast to the hippocampus, little has been known about how stress affects the amygdala and the nature of its role in the stress response. Recent evidence has demonstrated that the amygdala also plays a critical role in stress response and activation of the HPA axis (Le Doux 1994). It has been suggested that hippocampal plasticity mediates cogni-

tive aspects of behavioral impairments caused by stress, whereas changes in the amygdala are more likely to contribute to the affective aspects of stress disorders with memory consolidation.

2. Corticoid Receptor Dynamism and Corticoids

There are two types of receptors for corticosteroids: mineralocorticoid receptor (MR) and glucocorticoid receptor (GR). Both are ligand-dependent transcription factors. Of the two receptors, MR has a higher affinity for corticosterone/cortisol as well as aldosterone, whereas GR has a higher affinity for dexamethasone, a synthetic glucocorticoid, and approximately ten times lower affinity than MR for corticosterone (Joel and de Kloet 1994). The fact that corticosterone is an agonist with differential affinity for MR and GR has consequences for the relative occupation of MR and GR by corticosterone during the day. A low level of corticosterone found during the circadian nadir is already sufficient to activate most MR, while substantial activation of GR occurs with a high corticosterone level as seen at the circadian peak or after stressful events (Reul et al. 1987).

MR and GR belong to the steroid/nuclear receptor superfamily. All the receptors in the steroid/nuclear superfamily share common structural and functional domains: N-terminal transcription activation domain, central DNA binding domain, and C-terminal ligand binding domain (Beato et al. 1995; Kawata 1995). The activation of GR and MR is described as the nucleocytoplasmic trafficking model. A real-time imaging study using green fluorescent proteins (GFPs) showed that in the absence of a steroid GR is present in the cytoplasm (in an inactive form) (Kawata 2001; Kawata et al. 2001) (Fig.1). Steroid binding results in receptor activation and translocation to the nucleus. The inactive GR is found as a complex with other proteins that include heat-shock protein (HSP) 90 and HSP 70. These HSPs, interacting with steroid binding domains, facilitate the folding of receptor proteins into the appropriate conformation for the ligand binding (Couette et al. 1998; Yang and DeFranco 1996). The effects of HSP 90 association on GR trafficking were examined by using geldanamycin, which binds to HSP 90 and inhibits steroid binding affinity to GR (Nishi et al. 2001). The results were that COS-1 cell pretreatment with geldanamycin inhibits GR translocation from the cytoplasm to the nucleus by corticosterone, whereas in hippocampal neurons GR was translocated into the nucleus after the addition of corticosterone even when pretreated with geldanamycin, suggesting that the manner in which GR associates with HSPs for regulating ligand binding in hippocampal neurons might differ from that of COS-1 cells (Nishi et al. 2001).

Following ligand binding, HSP 90 and several associated proteins dissociate, and importin alpha binds to liganded GR. A mutant experiment in which the nuclear localization signal (NLS) was impaired on the GR clearly showed that the NLS is exposed when ligand binds to GR and is then recognized by importin alpha. Recent fluorescent resonance energy transfer (FRET) analysis indicated that liganded GR with importin alpha is directed into the nuclear pore of the nuclear membrane and

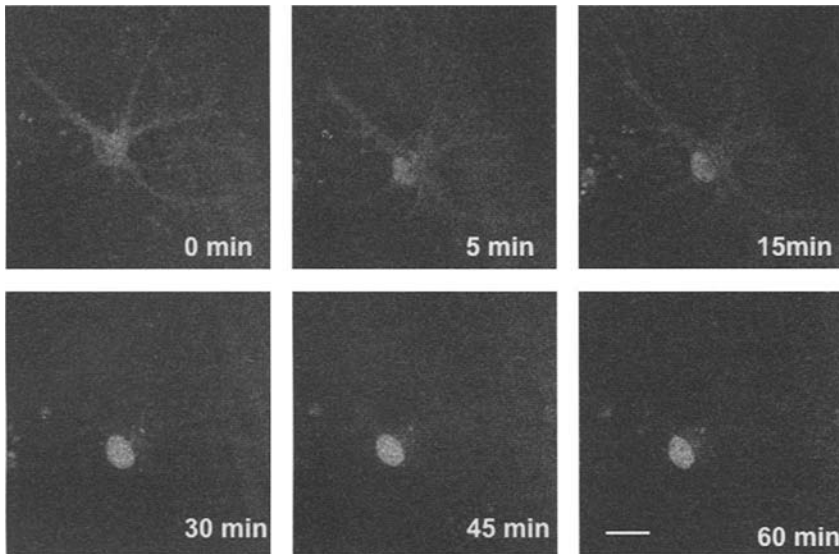


Fig. 1. Time-lapse imaging of green fluorescent protein- glucocorticoid receptor (GFP-GR) transfected hippocampal neuron. Corticosterone treatment induced GFP-GR translocation from the cell soma and neurites to the nucleus in a time-dependent manner. Bar: 10 μ m

liganded GR detached from importin alpha shortly after entering the nucleus (Tanaka et al. 2004). Importin beta, another subtype of the importin family, is still unknown to be involved in the GR transport into the nucleus, because little change was observed in the distribution of importin beta in spite of corticosterone-induced GR translocation to the nucleus, and FRET did not occur between liganded GR and importin beta (Tanaka et al. 2004). Pretreatment with microtubule disrupting agents, colchicine or nocodazole, did not cause any changes in dexamethasone-induced trafficking of GR into the nucleus, indicating that cytoskeletal tubulin is not, at least in part, involved in the translocation of the activated form of GR from the cytoplasm to the nucleus (Nishi et al. 1999). With regard to the ligand specificity, aldosterone, a specific agonist for MR, and progesterone, which has a chemical structure similar to aldosterone, induced nuclear accumulation of GR, but estradiol, which has a different structure at the ligand binding site, did not cause any translocation of GR. Signaling molecules such as cAMP-dependent protein kinase and MAP kinase did not affect the trafficking of GR into the nucleus by the treatment of forskolin and brain-derived neurotrophic factor (Nishi et al. 1999).

In the absence of ligands, MR resides both in the cytoplasm and in the nucleus. Agonists, corticosterone and aldosterone, increased the number of MRs residing in the nucleus. Analysis of a mutant of MR, in which the NLS is inactivated, demonstrated that the intact NLS is necessary for the trafficking of MR related to importin alpha, but not importin beta (Tanaka et al. 2005). MR has characteristics similar to

GR in terms of the interaction with cytoskeletal tubulin, signaling molecules, and ligands including progesterone and estradiol (Nishi et al. 2001).

3. GR and MR Interaction

By using immunocytochemistry in conjunction with specific antibodies of MR and GR, it was found that MR and GR are coexpressed in the same neurons of CA1/CA2 pyramidal and dentate gyrus cells of the hippocampal formation, parvocellular neurons of the hypothalamic nucleus, and neurons in the hypothalamic arcuate nucleus (Ito et al. 2000; Han et al. 2005).

Cotransfection experiments using CFP (cyan fluorescent protein)-GR and YFP (yellow fluorescent protein)-MR with quantitative analysis in COS-1 cells revealed that the nuclear accumulation rate of YFP-MR is faster than that of CFP-GR at a lower concentration of corticosterone, whereas at a higher concentration of corticosterone it is nearly equal. In contrast, hippocampal neurons exhibited no significant differences between rates of nuclear accumulation of CFP-GR and YFP-MR at either lower or higher concentrations of corticosterone (Nishi et al. 2001).

Many data have demonstrated that GR and MR regulate gene expression via binding to specific DNA sequences (glucocorticoid responsive element: GRE) in the promoter region of the gene by homodimerization: each homodimer binds to GRE. Recent FRET analysis by using *in vitro* hippocampal cultured neurons and COS-1 cells demonstrated the possibility of heterodimerization of GR and MR after ligand binding within the nucleus, not in the cytoplasm (Nishi et al. 2004). In the hippocampal formation, pyramidal neurons in the CA1/CA2 and granule cells of the dentate gyrus coexpress GR and MR (Ito et al. 2000; Han et al. 2005). Their relative concentrations and the ligand concentrations define the proportion of each receptor dimerization with its particular DNA-binding and transcriptional activities. It has been demonstrated that MR may predominantly form homodimers at a lower concentration of corticosterone, whereas at higher concentrations, which mimic stress conditions, the predominance of GR increases to promote the incidence of homodimerization. Our recent FRET analysis showed that the content of the GR-MR heterodimer was higher at higher concentrations of corticosterone than at lower concentrations. The existence of two types of corticoid receptors may allow a more flexible response to widely varying corticosteroid concentrations, giving the neurons the potential to provide a more finely orchestrated regulation of corticosteroid-responsive genes.

It has been demonstrated by immunocytochemistry and GFP imaging that in the nucleus GR as well as MR are distributed in a heterogeneous cluster formation (van Steensel et al. 1996; Htun et al. 1996; Nishi et al. 1999, 2001). Most of these clusters in the nucleus did not colocalize with newly synthesized RNA, RNA polymerase II, or splicing factor. FRAP (fluorescence recovery after photobleaching) analysis by visualizing the direct interaction of GR and GRE proposed a dynamic "hit-and-run" model in which the receptor undergoes continuous exchange between

chromatin regulatory elements and nuclear compartments (McNally et al. 2000).

4. Stress and Amygdala

The amygdala modulates memory consolidation with the storage of emotionally relevant information and is critical for the formation of long-term fear memory. Anatomically, the amygdala is composed of several distinct nuclear subgroups of cells: basolateral nucleus, central nucleus, and corticomedial nucleus. Among these nuclear subgroups, the basolateral nucleus has been paid the most attention because it receives sensory information from the thalamus and cortex and then forwards a signal to the appropriate target areas, which include the hippocampus, cortex, striatum, bed nucleus of the stria terminalis, and the central nucleus of the amygdala. Recent findings indicate that the adrenal stress hormones, epinephrine and corticosterone, converge to modulate memory consolidation (McGaugh and Roozendaal 2002). Norepinephrine released from the terminal of noradrenergic neurons in the nucleus tractus solitarius and glucocorticoids from the blood, affect through their receptors the neurons in the basolateral nucleus of the amygdala, influencing memory consolidation (Ferry et al. 1999). In particular, the activation of both beta and alpha adrenoreceptors in the basolateral nucleus of the amygdala is critical for mediating the noradrenergic influences on memory consolidation. Further studies indicate that *N*-methyl *D*-aspartate (NMDA) glutamatergic receptors, cAMP-dependent protein kinase A, and cAMP-response element-binding protein (CREB), regulate neurons in the basolateral nucleus of the amygdala for long-term memory formation and aversive conditioning (Schafe and LeDoux 2000; Josselyn et al. 2001).

Extensive evidence indicates that neurons in the basolateral nucleus of the amygdala contain GR as well as MR, and the low-affinity GR, not the high affinity MR, is involved in mediating corticosteroid effects on memory consolidation (Roozendaal et al. 1996). The effects of epinephrine and glucocorticoid on the consolidation of memory for emotional experiences are intimately linked to noradrenergic activation in the basolateral nucleus of the amygdala through direct involvement of glucocorticoid, as well as having an indirect effect via the nucleus tractus solitarius system (McGaugh and Roozendaal 2002).

Certain forms of chronic stress affect specific neuronal elements in the basolateral nucleus of the amygdala: chronic immobilization stress induces an enhancement of dendritic arborization of pyramidal and stellate neurons, whereas chronic unpredictable stress induces atrophy in bipolar neurons (Vyas et al. 2002). In addition, animals exposed to chronic immobilization stress continued to exhibit enhanced anxiety as manifested by a significant reduction in open-arm exploration and risk-assessment behavior in the elevated plus-maze even after 21 days of stress-free recovery (Vyas et al. 2004). At the cellular level, a persistent increase in the dendritic arborization of pyramidal neurons in the basolateral nucleus of the amygdala was observed (Vyas et al. 2004). Our single prolonged stress experiment, which is recognized as an animal PTSD model, induced the increase of dendritic arboriza-

tion of pyramidal neurons and also the higher number of nerve fibers of neuropeptide Y immunoreactivities in the basolateral nucleus of the amygdala (unpublished data). This stress-induced structural plasticity of the amygdala provides a cellular substrate for affective disorders that are triggered by chronic stress.

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Stress Vulnerability Induced by Neonatal Isolation and the Disturbance Between the Phosphorylation and Dephosphorylation of CREB

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1. Introduction

Lifetime prevalence of posttraumatic stress disorder (PTSD) has been found to range from 1.0% to 12.3% based on general-population and service-sector samples (Fairbank et al. 1995), which demonstrates that not all victims suffer from PTSD. It is conceivable that individual differences in stress reactivity may play a role in the development of PTSD. In addition, traumatization in early development, e.g., physical abuse or separation from parents, has been shown to be associated with the development of PTSD (Fairbank et al. 1995; Bremner et al. 1999; Pine and Cohen 2002).

Various animal studies indicate that early adverse experiences change stress reactivity in adult rats (Kaufman et al. 2000). Neonatal isolation as well as maternal separation affects the response of the hypothalamo-pituitary-adrenal (HPA) axis to acute stress in adult rats (Francis et al. 1999; Ladd et al. 1996; Plotsky and Meaney 1993). Furthermore, neonatal isolation induces an increase in the central noradrenaline drive and reduction in the activity of the inhibitory central GABA/BZ system in adulthood (Caldji et al. 2000; Liu et al. 2000).

Recent molecular pharmacological studies have revealed that while glucocorti-

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coids regulate neuronal gene expression through their intracellular receptors, neurotransmitters also modulate neuronal gene expression via the activation of protein kinases and transcription factors. Whereas homeostasis of the HPA axis and the neurotransmitter systems are maintained in adult rats that have been subjected to neonatal isolation, under a low-stress environment, it is plausible that an exposure to stress in adulthood may easily lead to alterations in neuronal gene expression through changes in the activity of transcription factors. Subsequently, stress vulnerability may become evident. Therefore, examining the influence of neonatal isolation on the phosphorylation and dephosphorylation of transcription factors in the adult rat brain may contribute to the elucidation of the molecular mechanism underlying stress vulnerability.

In this chapter, we describe the possible molecular mechanism for the stress vulnerability induced by neonatal isolation, based on the disturbance between the phosphorylation and dephosphorylation of cyclic AMP-response element binding protein (CREB), a major transcription factor. At first, we start with a brief description of neonatal isolation adopted in our study. Following this, we show the influence of neonatal isolation on the phosphorylation of CREB and the activity of protein phosphatases, key enzymes for dephosphorylation of phospho-CREB as well as auto-phosphorylated-calcium/calmodulin-dependent (CaM) kinases, in the adult rat hippocampus in response to a single restraint stress in adulthood.

2. Neonatal Isolation Paradigm

Pregnant female rats were housed individually in the breeding colony at constant room temperature ($23^{\circ} \pm 2^{\circ}\text{C}$) and humidity (60%) with a 12 h/12 h light/dark cycle (lights on 8:00 a.m.–8:00 p.m.). The litters were culled to 12 pups on postnatal day 1 (PN day 1).

Neonatal isolation was undertaken according to Kehoe's method with a minor modification (Kehoe and Bronzino 1999). Pups were isolated from the dam, nest, and siblings for a period of 1 h per day on PN days 2–9. Pups of the isolation treatment group were placed in individual round containers. The mothers and pups of the nonisolated group were left undisturbed until weaning. All litters were weaned on PN day 21, separated on the basis of sex, and maintained with ad libitum access to food and water. On PN day 90, both groups of rats were subjected to a single restraint stress for 45 min. Sham-treated rats were left undisturbed until weaning and were not subjected to the restraint stress in adulthood.

All animal procedures were in strict accordance with the Hiroshima University School of Medicine Animal Care Committee.

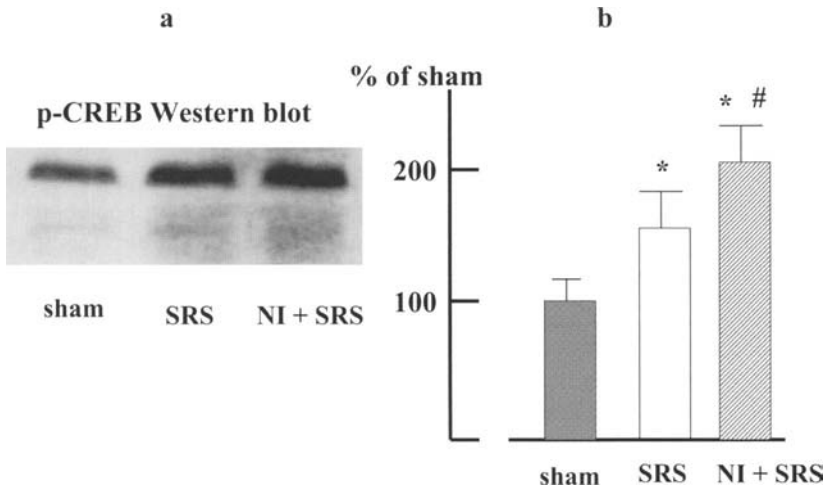


Fig. 1a,b. Western blot analysis of the hippocampal phospho-CREB expression in the rat subjected to single restraint stress (SRS) alone, neonatal isolation and single restraint stress (NI+SRS), or sham treatment. a Representative immunoblots for phospho-CREB in the hippocampus. b Comparison of the hippocampal levels of phospho-CREB in the sham, SRS, and NI+SRS groups. Results are expressed as the percentage of sham and are given as mean $\pm$ SEM ($n = 6$). Asterisk, $P < 0.01$ compared with the sham group; hash, $P < 0.01$ compared with the SRS group (one-way ANOVA with Fisher's test)

3. CREB Phosphorylation

Cyclic AMP response element binding protein (CREB) is phosphorylated at Ser133 by the activation of protein kinase A (PKA), calcium/calmodulin-dependent protein kinase IV (CaMK IV), and extracellular signal-regulated kinase (ERK) (Mathews et al. 1994; Waltereit and Weller 2003). After phosphorylation, phospho-CREB can regulate gene expression via its binding to the cAMP response element. It is well known that the activation of CREB phosphorylation by the increase in the levels of intracellular calcium induces the transcription of brain-derived neurotrophic factor (BDNF), closely involved in neuronal plasticity (Timmusk et al. 1993). In this context, we first examined the influence of neonatal isolation on the phosphorylation of CREB in adult rat hippocampus in response to an additional adulthood stress to elucidate whether the CREB-mediated gene expression might be associated with the molecular mechanism of the stress vulnerability. Western blot analysis for phospho-CREB, and CREB in the rat hippocampus was performed using our previous method (Fig. 1a) (Morinobu et al. 2000).

Western blot analysis revealed that the hippocampal levels of phospho-CREB of

adult rats subjected to neonatal isolation followed by a single restraint stress in adulthood (NI+SRS) as well as subjected to a single restraint stress (SRS) were significantly higher than those of adult rats subjected to the sham treatment (sham) (Fig. 1b). In addition, the hippocampal levels of phospho-CREB of adult rats subjected to NI+SRS were significantly higher than those of adult rats subjected to SRS (Fig. 1b). In these three groups of adult rats (sham, SRS, and NI+SRS), no significant differences were found in the levels of CREB in the hippocampus.

The results of our study suggest that the enhanced susceptibility of the alteration in hippocampal gene expression in response to adulthood stress is found in rats subjected to neonatal isolation. It is plausible that the change in hippocampal gene expression of individuals with early adverse experiences under a stressful environment may disturb the hippocampus function, such as learning and memory, and consequently, induce the onset of PTSD.

4. Serine/Threonine Phosphatase Activity

Protein phosphatases are key enzymes for dephosphorylation of phospho-CREB and autophosphorylated-CaM kinases. In contrast to CREB phosphorylation, the activation of protein phosphatase 1, through the enhancement of the serine/threonine phosphatase activity of protein phosphatase 2B (PP2B, calcineurin), dephosphorylates phospho-CREB, and reduces the rate of gene expression, such as *c-fos* or somatostatin (Lisman 1994; Stemmer et al. 1995; Bito et al. 1996). In addition, it is suggested that protein phosphatase 2A (PP2A) is associated with gene transcription mediated by the signaling complex of CaMK IV and CREB or of p70 S6 kinase (Westphal et al. 1998, 1999). For example, it is reported that PP2A plays an important role in the dephosphorylation of autophosphorylated CaM kinases, and therefore it is conceivable that the activation of PP2A may decrease the phosphorylation of CREB via the enhanced dephosphorylation of phospho-CaM kinase II and IV. Taken together, because changes in neuronal gene expression mediated by CREB phosphorylation in response to stress are involved in the pathophysiology of PTSD, it is postulated that the activity of protein phosphatases under a stressful environment also plays a role in the molecular mechanism of PTSD. In this context, we investigated the influence of neonatal isolation on the activity of protein phosphatases in the adult rat hippocampus in response to an additional adulthood stress. The level of serine/threonine phosphatase activity of protein phosphatases was quantitated as the amount of free phosphate derived from the synthetic phosphopeptide RRA(pT)VA (Promega, Madison, WI) by measuring the absorbance of molybdate-malachite green-phosphate complex as previously described (Takahashi et al. 2001; Morinobu et al. 2003).

PP2A activity assay revealed that the activity of PP2A in the hippocampus of adult rats subjected to NI+SRS as well as that of adult rats subjected to SRS was significantly higher than that of adult rats subjected to sham (Fig. 2a). However, no significant difference in the activity of PP2A was found in the hippocampus of adult

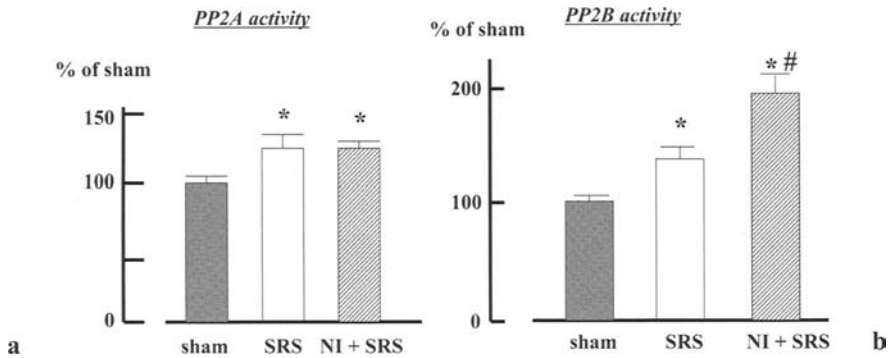


Fig. 2a,b. Analysis of the hippocampal PP2A and PP2B activity in the rat subjected to SRS alone, NI+SRS, or sham treatment. **a** Analysis of the hippocampal PP2A phosphatase activity in the rat subjected to SRS alone, NI+SRS, or sham treatment. **b** Analysis of the hippocampal PP2B phosphatase activity in rats subjected to SRS alone, NI+SRS, or sham treatment. Results are expressed as the percentage of sham and are given as mean $\pm$ SEM ($n = 8$, or 9). Asterisk, $P < 0.01$ compared with the sham group; hash, $P < 0.01$ compared with the SRS group (one-way ANOVA with Fisher's test)

rats subjected to NI+SRS and SRS (Fig. 2a).

PP2B activity assay revealed that the activity of PP2B in the hippocampus of adult rats subjected to NI+SRS and those to SRS was significantly higher than that of adult rats subjected to sham (Fig. 2b). In addition, the activity of PP2B in the hippocampus of adult rats subjected to NI+SRS was significantly higher than that of adult rats subjected to SRS (Fig. 2b).

The results of our study demonstrate that the activity of PP2B, but not PP2A, in the hippocampus of adult rats subjected to NI+SRS is significantly upregulated when compared with rats subjected to SRS, suggesting that PP2B may be more susceptible to NI than PP2A. Because the phosphorylation of CREB in the hippocampus of the NI+SRS rats is significantly increased, it is speculated that the decrease in the activity of PP2B may induce the reduction in the dephosphorylation of phospho-CREB in the NI+SRS rats. However, the result from the CREB phosphorylation in the NI+SRS rats is not in good agreement with this speculation. Although the precise mechanism of this discrepancy remains unknown, the enhanced phosphorylation of CREB mediated by PKA and CaM kinases may overcome the increased dephosphorylation of phospho-CREB mediated by PP2B in the hippocampus of rats subjected to NI+SRS.

On the other hand, the findings of significantly increased phosphorylation of CREB and significantly increased activity of PP2B in the hippocampus of rats subjected to NI+SRS indicate that the mobilization of intracellular Ca^{2+} in the hippocampus of rats subjected to NI is greatly increased in response to restraint stress. It

is well known that the intracellular Ca^{2+} regulates the activity of both CaM kinases and PP2B. Based on these findings, it is likely that the enhanced intracellular mobilization of Ca^{2+} in the hippocampus of the NI rats in response to stress is a key element of the stress vulnerability.

5. Stress Vulnerability Induced by Neonatal Isolation and Memory Function

The results of our study demonstrated that the phosphorylation of CREB and the activity of PP2B in response to adulthood stress were significantly upregulated in the hippocampus of rats subjected to NI. It is noteworthy that CREB as well as PP2B is closely involved in the formation of memory. For example, the induction of

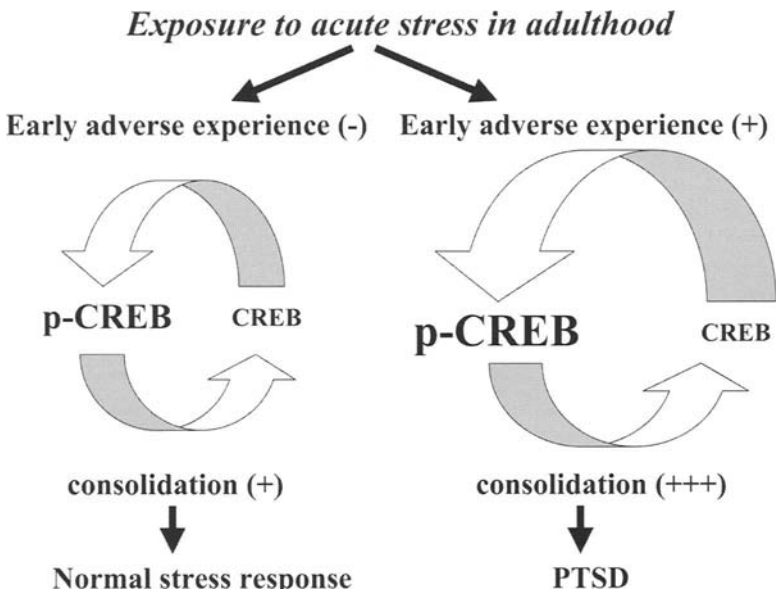


Fig. 3. A model of adulthood stress vulnerability induced by early adverse experiences. In individuals with early adverse experiences (*right*), an exposure to traumatic stress in adulthood induces an intense consolidation through the marked increase in the phosphorylation of cyclic AMP-response element binding protein (CREB), and leads to the development of post-traumatic stress disorder (PTSD). In contrast, in individuals without early adverse experiences (*left*), an exposure to traumatic stress also induces a consolidation to a lesser extent through the increase in the phosphorylation of CREB, and leads to a normal stress reaction

gene expression mediated by the CREB phosphorylation via PKA, and CaM kinases, plays an important role in memory acquisition and consolidation (Abel and Lattal 2001). Although the activation of PP2B leads to the extinction of fear memory (Genoux et al. 2002; Lin et al. 2003), calcineurin is also involved in the transition from short-term to long-term memory (Mansuy et al. 1998; Winder et al. 1998; Abel and Lattal 2001).

On the other hand, patients with PTSD often exhibit physiologic reactivity on exposure to cues that resemble an aspect of the traumatic event (Prins et al. 1995). It is conceivable that this type of reexperience of a traumatic event may be due to the abnormal memory function, such as the enhanced consolidation and dysfunctional extinction (Southwick et al. 2002; Rothbaum and Davis 2003). In this context, the enhanced phosphorylation of CREB and activation of PP2B in the hippocampus of rats subjected to NI in response to adulthood SRS suggest one plausible mechanism for the enhanced consolidation of stressful memory in individuals with early adverse experiences (Fig.3). However, the enhanced activation of PP2B in the hippocampus of the NI+SRS rats does not support the disturbed extinction of stressful memory often seen in patients with PTSD. Further investigations are required to elucidate the precise mechanism by which the dysfunctional memory in PTSD is caused by the disturbance between the phosphorylation and dephosphorylation of CREB in the brain.

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Neuroscience of Emotional Memory and Posttraumatic Stress Disorder

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Hironobu Iguchi⁵, and Keiko Takemoto⁵

1. Introduction

Posttraumatic stress disorder (PTSD) is now widely recognized in Japan following the Kobe earthquake and the Tokyo subway sarin poisoning in 1995. Wider awareness of this disorder has resulted in an increase in the number of patients presenting with the core symptoms of PTSD, which include: (1) persistent increased arousal; (2) reexperience of a traumatic event; (3) persistent avoidance of stimuli associated with the trauma; and (4) numbing of general responsiveness.

We have conducted studies to elucidate the biological mechanisms that cause these clinical symptoms and deeply engrain memories associated with highly emotional events (emotional memory). We used animal models to target a phenomenon called neurogenesis and the dynamics of a gene transcription factor called cyclic AMP-response element binding (CREB) protein in the hippocampus. This chapter discusses the results of some of our studies.

2. Memory and Plasticity in the Hippocampus

In 1990, Bergold et al. (1990) reported that learning in *Aplysia* was hindered by injecting the CRE-sequence into the nucleus of nerve cells, thus clarifying that learning in *Aplysia* requires binding of CREB to the promoter (CRE) region. Because mice with target mutations in the alpha and beta isoforms of CREB can survive,

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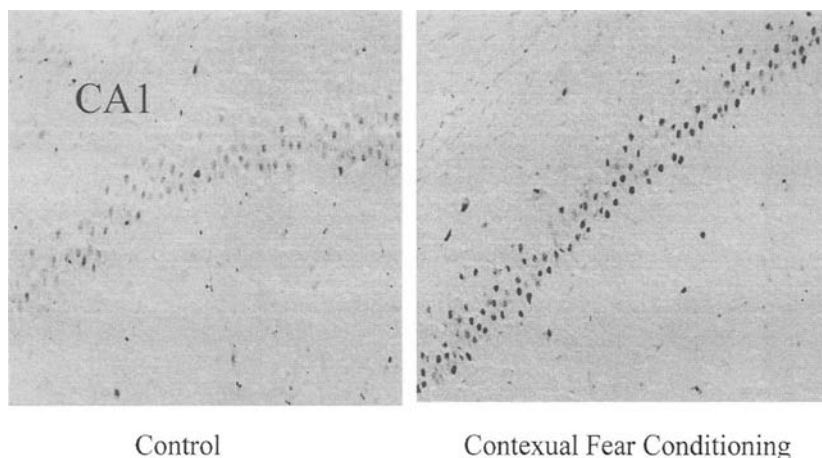


Fig. 1. Contextual fear conditioning (*right*) and pCREB immunopositive cells in CA1 region

they have been used to analyze CREB functions. These genetically altered mice exhibit impaired learning when examined using a contextual fear conditioning (CFC) paradigm and reduced long-term potentiation of the hippocampus, suggesting that CREB is necessary for hippocampus-dependent learning in mammals (Bourtchuladze et al. 1994). Furthermore, a study using genetically altered mice in which the LacZ gene was linked to the CRE region showed that LacZ was expressed in the hippocampus during hippocampus-dependent learning and in the amygdala during amygdala-dependent learning, thus clarifying that the expression of the genes involved in the CRE-CREB pathway is associated with memory formation (Impey et al. 1998). These gene expressions are thought to ultimately bring about changes in the shape of dendritic spines, thereby increasing transmission efficiency.

Long-term potentiation (LTP), or the physiological mechanism of memory, can generally be summarized as follows: signals enter postsynaptic nerve cells, and the level of intracellular cyclic AMP subsequently increases to phosphorylate CREB, which forms phosphorylated CREB (pCREB). This pCREB migrates to the nucleus and binds with the promoter region to begin production of nerve growth factors [e.g., brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), etc.]. These substances plasticize synapses into structures capable of LTP.

A histological study that directly and visually examined the involvement of CREB phosphorylation in learning had not previously been conducted. Therefore, we investigated the relationship between learning and CREB phosphorylation in the hippocampal CA1 region (Fig. 1) by assessing freezing behaviors in rats using a CFC paradigm, where the unconditioned stimulus was footshock and the conditioned stimuli were a shock cage and the surrounding environment.

3. Emotional Memory and CREB Phosphorylation in the Hippocampus

Differences in spatial memory in rats, due to factors such as sex and age, have been documented; therefore, experiments were developed to ascertain if changes in the number of pCREB immunoreactive cells in the hippocampal CA1 region correlate with learning. When compared with female rats, male rats were better at acquiring spatial memory, and a correlation was observed between the sexes for freezing behaviors and changes in the number of pCREB immunoreactive cells in the hippocampal CA1 region (Kudo et al. 2004). In addition, changes in the number of pCREB immunoreactive cells were limited to the hippocampal CA1 region.

Next, rats in two age groups (old rats 120 weeks of age, and young rats 12 weeks of age) were subjected to CFC, and at the same time, pCREB immunoreactive cells were stained to ascertain site specificity in the brain. The results showed that when compared with the old rats, young rats were better able to acquire spatial memory, and the number of pCREB immunoreactive cells in young rats increased in the hippocampal CA1 region. A correlation was also observed between the age differences in freezing behaviors and the changes in the number of pCREB immunoreactive cells in the hippocampal CA1 region (Kudo et al. 2005).

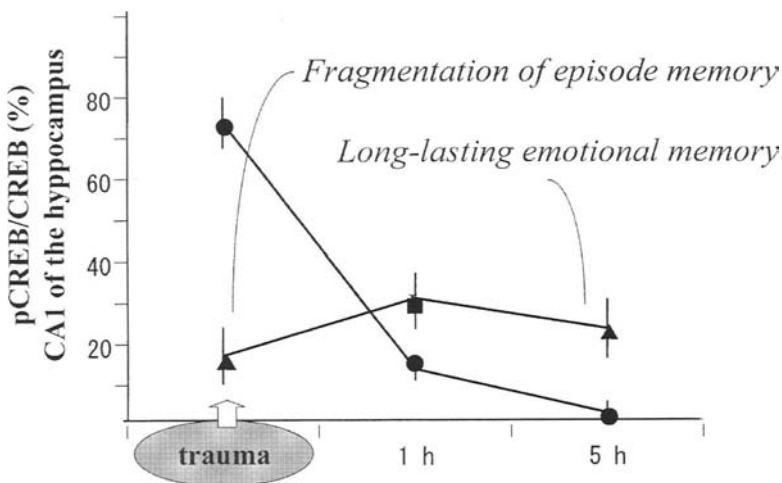


Fig. 2. Hypothetical molecular mechanisms of psychopathology of posttraumatic stress disorder. ●, control group; ▲, footshock group

The above results indicate that protein synthesis in the hippocampal CA1 region is involved in the accumulation and maintenance of spatial memory driven by fear. Rats that received electric shocks at increasing intensity were found to have a significantly lower number of pCREB immunoreactive cells immediately following an electric shock, regardless of intensity, when compared with a control group that did not receive electric shocks. Once the severity of the footshock (intensity of electric current) reached a certain level, the number of pCREB immunoreactive cells decreased in the hippocampus; however, after a certain period of time, the number of pCREB immunoreactive cells began to increase and reach a very high level, and, during this period, marked freezing behaviors were observed. Further investigations are necessary to clarify the cause of this change in the number of pCREB immunoreactive cells. This phenomenon may be associated with PTSD-related symptoms. We suspect that the rapid decrease in the number of pCREB immunoreactive cells is involved with memory fragmentation immediately after a traumatic event and that the subsequent increase is involved with long-term deep engraving of the emotional event (Fig. 2).

4. Discovery of Neurogenesis

For a long period of time, it was believed that production of nerve cells in the mammalian brain was limited to the embryonic stage. Thus, some recovery from permanent nerve cell and tissue damage due to trauma or aging was attributable to remaining cells forming new nerve connections. Altman and Das (1965) were the first to report the possibility of neurogenesis. They performed [^3H]-thymidine autoradiography, which is capable of labeling newly formed cells, and reported the existence of progenitor cells capable of differentiating into neurons or glia in the dentate gyrus and olfactory bulb of adult rats. This report did not have much initial impact; however, after more than 30 years, techniques that can easily identify neurogenesis are now available, and studies have documented that neurogenesis is seen in the brains of adult birds, rodents, monkeys, and humans, and that progenitor cells can mature to adult neurons and carry out normal neuronal functions. Besides the hippocampus, recent studies have reported neurogenesis in the neocortex of primates, including monkeys and humans (Gould et al. 2001).

The regulation of neurogenesis involves several different stages, including proliferation, differentiation, and survival (Malberg et al. 2000). The number of nerve cells produced in the dentate gyrus is lower than the number of cells in the granular cell layer (1–2 million), and, in rats and mice, only 1000–3000 cells are produced daily. However, because neurogenesis occurs throughout life, the cells in the dentate gyrus account for 10%–20% of the total. Furthermore, it has been suggested that newly formed cells in the dentate gyrus are involved more preferentially in processing the latest information (van Praag et al. 2002).

5. Neurogenesis and Stress Vulnerability

When rats and monkeys are exposed to a stressful event, neurogenesis decreases in the dentate gyrus (Gould et al. 1997, 1998). A change in cellular proliferation occurs in rats if they smell the scent of a predatory animal, such as a fox, and the same occurs in monkeys if they are under social stress. An experiment was conducted using male adult rats with intact adrenal glands and male adult rats that underwent bilateral adrenalectomy and low-dose corticosterone (endogenous glucocorticoid) supplementation. Both groups of rats were then subjected to the scent of a fox. The results showed that smelling the scent quickly lowered the degree of cellular proliferation in the dentate gyrus, but this type of decrease was not seen with other scents. This decrease was transient and dependent on increased stress-induced adrenal hormones, and there was no significant intergroup difference 3 weeks later. In this manner, adrenal hormones appear to suppress the production of new granular cells throughout life by suppressing the proliferation of progenitor cells. Also, when rats were subjected to a daily, 1-h traumatic stress for 28 days, the level of cortisol was continuously high, neurogenesis decreased, and the volume of the granular cell layer decreased.

In pups born from rats that were subjected to an immobilization stress late in the gestation period, the neurogenesis in the hippocampal dentate gyrus was low throughout their lives, and impaired hippocampus-dependent learning was confirmed (Lemaire et al. 2000). Many PTSD patients have experienced childhood stress, such as abuse, suggesting that childhood stress affects neurogenesis, and ultimately creates PTSD susceptibility.

However, our research indicates that in adult rats raised in a favorable environment during the growth period, neurogenesis in the hippocampal dentate gyrus increases significantly after a stressful event (manuscript in preparation). These findings suggest that childhood experiences and environments, depending on their quality, can affect adulthood neurogenesis by creating not only vulnerability, but also resilience to PTSD.

6. Conclusion

Experiments were developed to count the number of phosphorylated cyclic AMP-response element binding (pCREB) protein positive cells. pCREB immunoreactive cells in the hippocampal CA1 region correlated with emotional learning. The results indicate that protein synthesis in the hippocampal CA1 region is involved in the accumulation and maintenance of spatial memory driven by fear. However, rats that received electric shocks at increasing intensity were found to have a significantly lower number of pCREB immunoreactive cells immediately following an electric shock, when compared with a control group that did not receive electric shocks. We suspect that the rapid decrease in the number of pCREB immunoreactive cells is

involved with memory fragmentation immediately after a traumatic event and that the subsequent increase is involved with long-term deep engraining of the emotional event.

Other research by our group indicates that in adult rats raised in a favorable environment during the growth period, neurogenesis in the hippocampal dentate gyrus increases significantly after a stressful event. These findings suggest that childhood experiences and environments, depending on their quality, can affect adulthood neurogenesis by creating not only vulnerability, but also resilience to PTSD. In the future, neuroscience will continue to advance at a remarkable pace. In support of such academic advancement, it will be necessary to longitudinally examine the effects of the genes and environmental factors involved in the formation of the brain, and, in particular, the cytoarchitecture of molecules, nerve cells, and neural circuits. It will then be necessary to ascertain the causal relationships, from early neurological development to adulthood, between these factors and psychiatric symptoms. Clarification of these difficult questions will aid in solving the greatest mystery surrounding the brain and mind.

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Studies on Pathophysiology of PTSD Using the SPS Model

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1. Introduction

Animal models are important tools for the study of pathophysiology and the development of new treatments for mental disorders. A number of animal models of post-traumatic stress disorder (PTSD) have been proposed, and these can be categorized into two major groups according to their basic concepts. One idea of model construction is that stress given to animals should simulate traumatic events in humans. The researchers utilize social stress which is more naturalistic than electric shocks or restraint (Koolhaas et al. 1997). For example, animals are exposed to a predator or predator odor and the alterations induced by the stress exposure are analyzed. Pathophysiological consequences of predator stress are described by Adamec in Chap. 5.

In the other animal models of PTSD, researchers focus on the alterations induced by stress resembling symptoms of PTSD, although the stress paradigm itself is completely artificial. As was reviewed by Foa et al. (1992), animals exposed to uncontrollable and unpredictable stress showed similar disturbances to those found in PTSD. Because pathogenesis of mental disorders is still unclear, most animal models are based on similarities in neurochemical, neurophysiological, or behavioral manifestations between model animals and clinical observations. However, “face validity” is only the first step in establishing a model (Yehuda and Antelman 1993). Therefore, although animal research simplifies the investigation of possible pathophysiology and molecular mechanisms of human diseases, confirmation in clinical research is needed.

2. Single Prolonged Stress as a PTSD Model

The rats exposed to single prolonged stress (SPS), consisting of a 2-h restraint fol-

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lowed by 20 min of forced swimming and ether anesthesia, indicated enhanced inhibition of the hypothalamus-pituitary-adrenal (HPA) system 1 week after the stress exposure (Liberzon et al. 1997). In addition, the expression level of glucocorticoid receptor (GR) was increased in the hippocampus of the SPS rats, which should be a possible cause of facilitated feedback regulation of the HPA axis (Liberzon et al. 1999). Although its pathophysiological significance still remains elusive, such an alteration in the HPA response is most reproducibly observed in PTSD patients (Yehuda 2001). Here we describe our further characterizations of the SPS rats, which were done in collaboration with Drs. Harada, Yamaji, and Matsuoka in the Department of Neuroscience, Medical Biology Research Laboratories, Fujisawa Pharmaceutical Co. Ltd.

3. Hippocampal Dysfunction of SPS Model

Hippocampal dysfunction has been suggested in the patients of PTSD based on the following findings: (1) dysregulation of the HPA axis (Sapolsky et al. 1984; Yehuda 2001), (2) deficits in declarative memory (Bremner et al. 1993, 1995; Barrett et al. 1996; Jenkins et al. 1998), and (3) reduced volume of the hippocampal formation (Pitman et al. 2001). We investigated hippocampal synaptic plasticity and hippocampus-dependent behavioral learning in SPS rats (Kohda et al. 2002). Interestingly, the SPS-induced alterations of synaptic plasticity in the CA1 region followed a biphasic profile in their time course after the stress exposure. Long-term depression (LTD) was impaired just after SPS stress exposure, while long-term potentiation (LTP) seemed intact. Although LTD recovered 1 day after SPS, both LTP and LTD were attenuated again 1 week later. The results of the Morris water maze test, a hippocampus-dependent spatial learning paradigm, were well correlated with the profile of hippocampal synaptic plasticity; no difference 1 day after SPS, but significant impairment 1 week later. We could not find increased neuronal death in the hippocampus in the SPS rats at all time points investigated. These findings suggested that gross anatomical changes were not necessary for stress-induced hippocampal dysfunction. There have been controversies over the causal relationship of the smaller hippocampal formation in PTSD patients. Our findings supported the idea that the smaller volume of hippocampus was not the outcome of the traumatic experience. However, it is necessary to study the long-term effect of SPS on the hippocampus in order to rule out the possibility that SPS may finally result in hippocampal damage.

4. Fear Memory in the SPS Model

The neuronal mechanisms underlying fear conditioning and failure of its extinction were suggested to be involved in the production of reexperiencing symptoms, such

as intrusive memory, in PTSD (Charney et al. 1993). Harada et al. (2004) indicated that contextual fear conditioning was enhanced in rats 1 week after the SPS (the 1-week SPS rats). Although this result is intuitively acceptable, it should be noted that contextual fear conditioning was demonstrated to be dependent on the hippocampus (Kim and Fanselow 1992; Phillips and LeDoux 1992) and that the 1-week SPS rats clearly showed malfunction of the hippocampus as described above. Previously, it was reported that *Nf1^{+/-}Nmdar1^{+/-}* mutant mice, which had dissociation in hippocampus-dependent spatial learning and contextual fear conditioning, utilized the different strategy in context learning (Frankland et al. 1998). In learning a context, normal mice are supposed to use hippocampus-dependent polymodal cues, whereas *Nf1^{+/-}Nmdar1^{+/-}* mutant mice seemed to detect specific unimodal cues, probably compensating their hippocampal dysfunction. The 1-week SPS rats in which the same dissociation was observed may also change the strategy in context learning. To test this possibility, we performed a fear discrimination test. If the SPS rats changed the learning strategy, they should also indicate enhanced freezing in the fear discrimination test. This means that the SPS rats did not learn a context in a hippocampus-dependent manner, even though contextual fear conditioning was enhanced. The 1-week SPS rats well distinguished the contexts in fear discrimination, which indicated that there was no compensation of the hippocampal functional deficits by changing the strategy in context learning (Kohda et al. 2004).

Then, why was contextual fear conditioning enhanced in the 1-week SPS rats? Our study on synaptic plasticity in the cortical input to amygdala in the slice preparation revealed that SPS persistently suppressed LTP in the external capsule (EC)-lateral amygdala (LA) synapses (Kohda et al. 2004). It was reported that, via the activation of GABAergic interneurons, cortical afferents to basolateral amygdala, which includes LA, controlled projection neurons by feed-forward inhibition or excitation followed by inhibition (Lang and Pare 1997, 1998; Rosenkranz and Grace 2002). Impaired LTP in the EC-LA synapses in the 1-week SPS rats possibly resulted in disinhibition of pyramidal projection neurons in LA. The inputs from the medial prefrontal cortex (mPFC) strongly inhibited BLA neurons (Rosenkranz and Grace 2001, 2002) and disinhibition of LA and inactivation of prefrontal cortex reportedly enhanced contextual fear conditioning (Sacchetti et al. 2002; Isoardi et al. 2004). Thus, disinhibition of LA neurons through EC-LA LTP suppression explains enhanced contextual fear conditioning in the 1-week SPS rats. This explanation is in accordance with recent brain imaging data. Structural and functional abnormalities were found in the mPFC of PTSD, which resulted in failure of inhibition of amygdala (Bremner et al. 2003; Liberzon and Phan 2003; Yamasue et al. 2003; Shin et al. 2005). Further electrophysiological investigations in vivo will be necessary to clarify the interactions between mPFC and amygdala in the SPS rats.

5. Conclusion

The SPS rats clearly indicated “face validity” as a model of PTSD in several as-

pects. Thus, studying pathophysiology of the SPS model will contribute to clarifying the molecular and cellular mechanisms of PTSD. In fact, we found that mitogen-activated protein kinase phosphorylation was suppressed in the LA, whereas there was no significant alteration in the hippocampus (Kohda et al. 2004). Harada et al. (2004) showed that blockade of GR prior to SPS exposure inhibited enhanced contextual conditioning in the 1-week SPS rats. Further studies will give us clues for novel pharmacological treatments of PTSD. However, it must always be kept in mind that a model simulates only several aspects of an actual disorder. To avoid the overuse of analogies, close interaction between animal research and clinical research is necessary.

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Mechanisms of Lasting Change in Anxiety Induced by Severe Stress

Robert Adamec, Jacqueline Blundell, Kirby Strasser, and Paul Burton

1. Introduction

Affective disorder may arise from exposure to severe stress (Davidson et al. 2002; Holsboer 2001; Keck and Holsboer 2001; Petty 1995; Shalev et al. 1998). Stress precipitation of affective disorder is a serious problem given that 50%–60% of North Americans experience traumatic stress in their lifetimes (Kessler et al. 1995), and of those 15% may develop posttraumatic stress disorder (PTSD) (Kessler et al. 1994).

There are promising animal models of lasting effects of stress on affect. Prominent among them is classical fear conditioning of freezing. Studies of this form of learning have uncovered mechanisms of neural plasticity underlying acquisition and extinction of enhanced fear response to simple and complex sensory stimuli (contextual conditioning) (Blair et al. 2001; Maren et al. 1994; Rogan et al. 1997; Schafe et al. 2001). There is clinical interest in classical fear conditioning as a model of mechanisms of onset of PTSD to inform poststressor prophylactic intervention in humans (Pitman et al. 2002), and to suggest means to improve therapeutic outcome of exposure therapies (Myers and Davis 2002). Neural plasticity and long-term potentiation (LTP) in amygdala circuitry contribute to both conditioning and extinction of fear (Bauer et al. 2001; Blair et al. 2001; Schafe et al. 2000; Schafe et al. 2001; Schafe and LeDoux 2000a; Myers and Davis 2002; Nader et al. 2000a). Moreover, these forms of learning are *N*-methyl-D-aspartate (NMDA) receptor dependent (Baker and Azorlosa 1996; Campeau et al. 1992; Davis 2002; Decola et al. 1991; Fanselow et al. 1992; Kim et al. 1991; Lee and Kim 1998; Lee et al. 2001; Stiedl et al. 2000), as are some but not all forms of amygdala neural plasticity associated with acquisition and extinction of fear conditioning (Bauer et al. 2002; Davis 2002; Goosens and Maren 2002; Lee et al. 2002; Royer and Pare 2002; Tsvetkov et al. 2002).

However, fear conditioning cannot model all affective disorders associated with severe stress. Sensitized fearfulness manifested as generalized anxiety is also a feature of PTSD (Pitman 1997). Animal models of such changes, therefore, are also

relevant to stress precipitation of affective disorder. Moreover, animal models with ecological validity with regard to stressors are important to both validate and extend the findings with conditioning models.

Studies of lasting changes in affect following exposure to species-relevant life-threatening circumstances provide models of stress-induced affective psychopathology with ecological validity. Exposure of rodents to predator stimuli (predator stress) is fear provoking and stressful (Adamec et al. 1998; Blanchard et al. 1998; Dielenberg et al. 2001; McGregor et al. 2002). Moreover, predator stress (exposure to a cat) lastingly increases rodent anxiety when the exposure is unprotected and inescapable (Adamec and Shallow 1993; Adamec 1997). Behavioral changes are detectable in several tests of anxiety, including elevated plus maze (EPM), light-dark box, social interaction, and acoustic startle (Adamec 2003).

2. Predator Stress as a Model of Aspects of PTSD

Lasting changes in behavior may be produced with a brief (5-10 min) unprotected exposure of rats to a cat. Cat exposure creates a species-relevant life-threatening experience, which rats recognize as threatening both in their behavior and pattern of neuroendocrine response (Adamec 1997; Adamec et al. 1998). Therefore, predator stress has ecological validity in that it mimics brief, intense, life-threatening experiences with lasting affective consequences. Moreover, path analysis reveals that the intensity of the experience measured as cat attack and rat defensive reaction to that attack is directly predictive of degree of anxiogenic-like affective change in behavior (Adamec et al. 1998). Prediction of symptom severity by the nature of the stress experience has been reported in the clinical literature (Schnyder et al. 2001; Ikin et al. 2004). Dose response like clinical effects are also mimicked in this model by the reduced impact on rodent affect by exposure to predator odors as compared with unprotected exposure to a cat (Adamec et al. 1997).

The fact that not all traumatically stressed humans develop PTSD points to differential vulnerability to stress. Rats also show differential vulnerability to predator stress (Cohen et al. 2003).

With regard to lasting consequences of predator stress, behavioral and stress hormone changes induced by predator stress may model aspects of both core and generalized affective symptoms (Adamec 1997; Adamec et al. 1998). One aspect is the duration of symptoms. If one takes duration of effect as a ratio of life span, then changes produced by predator stress lasting 1 week or more last long enough to be considered a model of chronic symptoms. For example 1 week in a 3-year life span of a rat (0.64%) is proportional to 5.5 months of a person living 72 years (Adamec 1997; Adamec et al. 1998).

Another aspect of PTSD is avoidance of trauma reminders. It has been suggested that avoidance of open spaces (avoidance of the open arms of the EPM) following predator stress may be in part an avoidance of open spaces reminiscent of the open space (large room) in which the cat exposure takes place (Adamec 1997; Adamec et

al. 1998).

Hyper arousal aspects of PTSD may be modeled by the increases in acoustic startle amplitude and delay in habituation of startle persisting at least 1 week after predator stress. Similar changes in startle amplitude and habituation are seen in PTSD patients (Adamec 1997). Persistent anxiety-like behavior is another model for hyper arousal aspects of PTSD and appears in a variety of measures of rodent anxiety following predator stress.

Reexperiencing in the form of enhanced stress response to trauma reminders might also be modeled by the following findings. In this study, hooded rats were either handled as is usually done (control), or exposed to a cat for 5 min ($n = 30$ per group; see Adamec and Shallow 1993 for methods of treatment). One week after treatment, rats were tested in the EPM for 5 min as described elsewhere (Adamec and Shallow 1993). After a wait period of either 15 min or 24 h, rats were killed and

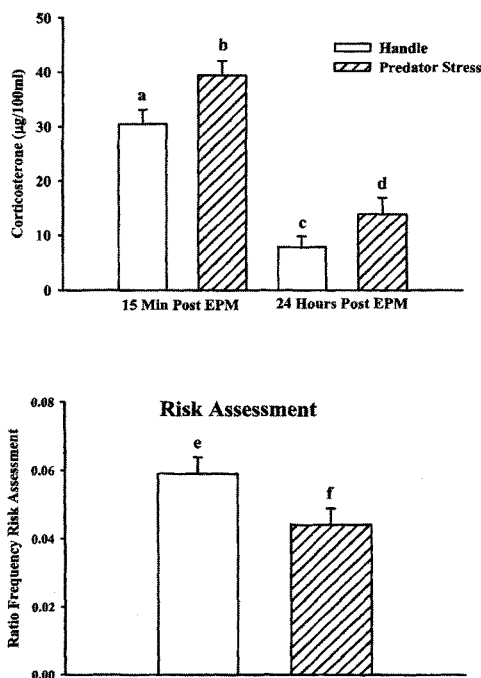


Fig. 1. Plotted in the *upper panel* are mean + SEM of plasma corticosterone taken from controls (handle) and predator-stressed rats. Trunk blood was taken from separate groups of rats either 15 min or 24 h after 5 min of free exploration of an elevated plus maze (EPM). Testing in the EPM took place 1 week after handling or predator stress. Means marked with different letters differ from each other. These differences are based on the time of sampling of blood (15 min or 24 h) and stress (handle or predator stress) as the main effects (see text). Plotted in the *lower panel* are mean + SEM of risk assessment in control and stressed rats. These means differ from each other by *t* test (see text)

trunk blood taken for stress hormonal analysis (see Adamec et al. 1998 for methods). Plasma levels of corticosterone were assayed in duplicate using commercial RIA kits in which intraassay and interassay variability was less than 7% as described elsewhere (Adamec et al. 1998). Data were analyzed with analysis of variance assessing stress (handle, predator stressed) and time of sampling of blood (15 min or 24 h) effects. There were main effects of stress and time of sampling on levels of corticosterone ($F(1,56) = 5.10, P < 0.03$; $53.08, P < 0.001$, respectively) and no interactions. As illustrated in Fig. 1, predator-stressed rats showed greater levels of corticosterone following exposure to the EPM than handled controls at both sampling times. In addition, hormonal levels in both groups had diminished 24 h after EPM exposure. Moreover, predator-stressed rats in this study were more anxious in the EPM. As an example, the decrease in risk assessment normally seen following predator stress was shown by rats in this study (Fig. 1; $t(56) = 2.18, P < 0.05$ comparing handled and predator-stressed rats). Risk assessment was measured as the frequency of stretch-attend explorations of the open arms with hindquarters in a closed arm as a ratio of time in the closed arms.

Handled rats showed higher levels of corticosterone at 15 min posttest than 24 h posttest, consistent with the stressful nature of plus maze exposure (Pellow et al. 1985). Of particular interest is the fact that at 15 min after EPM exposure, predator-stressed rats displayed an enhanced stress hormonal response. This enhanced stress hormonal response has its parallel in PTSD patients who show elevated cortisol response to non-trauma-related stressors (Butler 1989). If the open arms of the EPM are reminiscent of open spaces associated with predator stress, then enhanced stress hormonal response of stressed rats may represent an enhanced stress response to trauma reminders, as has also been reported in human PTSD (Elzinga et al. 2003).

Elevation of corticosterone in stressed rats relative to controls at 24 h likely reflects the persisting increase in resting corticosterone levels following predator stress seen in previous studies (Adamec 1997; Adamec et al. 1998). The persisting elevation of basal corticosterone in predator-stressed rats does not model the reported decreases in cortisol levels in PTSD sufferers (Yehuda et al. 2000). However, rat strain may be a factor in these levels. Predator stress increases and tends to decrease basal corticosterone levels in predator-stressed hooded and Wistar rats, respectively (Adamec 1997). Moreover, when stressed but not PTSD sufferers are compared with stressed PTSD sufferers, the decreased levels of resting cortisol may not be seen (Bonne et al. 2003; Seedat et al. 2003). In some cases, elevated levels of basal cortisol (evening) are reported in women victims of partner violence with PTSD (Pico-Alfonso et al. 2004). Finally, a recent large community study concluded that neither trauma per se nor PTSD led to sustained increases or decreases in mean urinary cortisol. However, PTSD was associated with higher catecholamine levels (Young and Breslau 2004). It is possible that neuroendocrine response is complexly related to stress and victim characteristics making generalizations difficult.

3. Neural Mechanisms of Effects of Predator Stress

3.1. NMDA Receptor Dependence of Neurobehavioral Changes Produced by Predator Stress

There are parallels between neural mechanisms underlying fear conditioning and neural mechanisms underlying behavioral effects of unprotected predator stress. Predator stress effects on behavior are NMDA receptor dependent (Adamec et al. 1999a,b). Systemic administration of competitive and noncompetitive NMDA receptor blockers 30 min before, but not 30 min after, predator stress prevents lasting increases in rodent anxiety. Local NMDA receptor block in the amygdala also prevents increased rodent anxiety (Adamec et al. 1999b). The observation that injection prior to stress, but not after, prevents behavioral change is consistent with the hypothesis that NMDA-dependent long-term potentiation (LTP) underlies behavioral changes.

3.2. Relationship of Brain Changes to Behavioral Changes Produced by Predator Stress

A body of evidence in two species suggests that severe stress produces NMDA-dependent LTP of amygdala afferent and efferent transmission, which underlies specific behavioral changes. In the cat, pharmacological stress induced by the anxiogenic benzodiazepine inverse agonist, FG-7142 (*N*-methyl- β -carboline-3-carboxamide), induces lasting increases in defensive responsiveness, which are closely associated with LTP of excitatory transmission from amygdala to lateral periaqueductal gray (PAG) (Adamec 1993, 1997, 1998a, b, c). LTP appears initially in both hemispheres. However, LTP is longer lived in the right amygdalo-PAG pathway, and, unlike the left hemisphere, persists as long as the behavioral changes. FG-7142-induced LTP and behavior changes in cats are NMDA receptor dependent (Adamec 1998a, b, c). Moreover reversal of right amygdalo-PAG LTP with low frequency stimulation (LFS) selectively reduces defensive behavior in cats (Adamec 1999). LFS does not affect predatory behavior, suggesting a specificity to defensive behavior of LTP in this pathway. In a parallel experiment, LFS of PAG also reverses anxiogenic effects of predator stress in rats (Adamec 2001).

Recent electrophysiological studies in rodents suggest that predator stress leads to LTP-like changes in transmission over several amygdala afferent and efferent pathways (Adamec et al. 2001). As in the cat, these LTP-like changes are NMDA receptor dependent (Blundell et al. 2005a). Moreover, like in the cat, stress-induced LTP in specific amygdala pathways in rodents may mediate specific behavioral changes. Path analysis implicates LTP in the right ventral angular bundle (VAB) input to the basolateral amygdala (BLA) in anxiogenic effects of predator stress in

the EPM (Adamec et al. 2001). In studies examining the relationship of VAB–BLA transmission at 9 days after predator stress, changes in transmission in both hemispheres were predictive of plus maze anxiety. In this instance, LTP in the right hemisphere and long-term depression (LTD) in the left hemisphere created by predator stress were the predictors. Of interest are more recent findings that this left hemisphere LTD appears to fade by 11 or 12 days after predator stress (Adamec et al. 2005b). As discussed below, this change appears to alter the circuitry that comes to control behavioral change over time.

Subsequent studies examined the predictive role of amygdala efferent transmission in rodent response to predator stress. Single-pulse stimulation of central amygdala (ACE) evokes a short latency negative going field potential in the lateral column of the PAG (Adamec et al. 2001, 2003). The PAG recording site receives monosynaptic projections from the ACE (Rizvi et al. 1991), which have mixed excitatory and inhibitory effects on single PAG cells (Da Costa Gomez and Behbehani 1995). Moreover, a body of evidence implicates ACE and PAG in rodent defensive and anxiety-like behaviors (Brandao et al. 1999; Davis 1990, 1992; Kemble et al. 1990; Kopchia et al. 1992; Moller et al. 1999; Rosen and Davis 1990; Yamashita et al. 1989), as well as in stress-induced increase in rodent anxiety (Makino et al. 1999; Shepard et al. 2000; Swiergiel et al. 1992), and PAG is activated by predator stress (Canteras and Goto 1999). In addition, information flow between ACE and ventrolateral PAG is involved in expression of contextually conditioned fear (freezing) (Carrive et al. 2000). Finally, glutamatergic mechanisms in PAG contribute to increased rodent anxiety (Brandao et al. 1999).

Initial work revealed that predator stress increased the size of the ACE–PAG evoked potential 1 day after predator stress in both hemispheres, suggesting a LTP-like change in ACE–PAG transmission (Adamec et al. 2001). When ACE–PAG transmission is examined 9 or 11–12 days after predator stress, LTP is present in the right but not in the left hemisphere (Adamec et al. 2001, 2003). This finding parallels effects of FG-7142 on amygdalo–PAG transmission in the cat mentioned above.

Path analysis at 10 or 11 days poststress revealed that variation in right VAB–BLA and right ACE–PAG transmission over control and predator stressed accounts for all of the variance in plus maze anxiety (open arm avoidance and risk assessment) (Adamec et al. 2005b). In light of the original path analyses at 9 days after stress, these data suggest a shifting of control of behavioral change in plus maze at least from bihemispheric to right hemispheric.

The high degree of prediction at 10 or 11 days was achieved by constructing a right limbic transmission latent variable (factor analysis) with VAB–BLA and ACE–PAG transmission measures as variables loading on the factor. This model suggests shared transmission variance within these two pathways is highly predictive of behavioral variation. Two modes of influence are suggested; parallel and linear. A linear process would involve sequential effects from VAB>BLA>ACE>PAG. In this model ACE>PAG represents efferent influence to behavior. A test of this model involved using ACE–PAG transmission as a covariate to determine if it alone could eliminate the differences between control and stressed rats. This proved to be the

case for risk assessment but not open arm avoidance in the EPM (measured as ratio time or the ratio of open arm time to total time in any arm of the maze and ratio entry or ratio of open arm entries to total arm entries) (Adamec et al. 2005b). ACE–PAG transmission similarly accounted for predator stress-induced increases in startle amplitude (Adamec et al. 2003) and delay of habituation of startle (Blundell et al. 2005a).

In contrast, transmission changes in VAB–BLA and ACE–PAG pathways do not seem to account for effects of predator stress on social interaction or light–dark box behavior. There are several reasons for this conclusion. First, predator stress effects on social interaction (increased withdrawal from partner or decreased time of social interaction) are not NMDA receptor dependent (Blundell et al. 2005b). The same dose of the NMDA receptor blocker CPP given before predator stress, which blocks LTP in VAB–BLA and ACE–PAG, is without effect on social interaction. CPP, however, does prevent increased predator stress-induced lighted chamber avoidance in the light–dark box test (Blundell et al. 2005b), but LTP in VAB–BLA and ACE–PAG pathways has been observed without changes in light–dark box behavior (Adamec et al. 2005b).

Findings in humans provide some neurobiological face validity to the study of neural plasticity in amygdala circuitry to gain insights into mechanisms of PTSD-induced affective disorder. Brain imaging studies have implicated the anterior temporal lobe (Shin et al. 1997), and in particular hyperexcitability of the right amygdala in response to script-driven trauma reminders in PTSD patients (Rauch et al. 1997a, b; Rauch and Shin 1997; Shin et al. 1997; Shin et al. 1999).

3.3. Molecular Pathways Involved in Brain and Behavioral Changes

Predator stress effects on expression of phosphorylated cyclic AMP response element binding protein (pCREB) have been examined as a marker of initiation of neuroplastic change of long duration. Because phosphorylation of CREB is regulated by NMDA receptors (Das et al. 1997), it was hoped that visualization of pCREB would be helpful in identifying putative neural circuitry mediating behavioral change in which NMDA-dependent late-phase LTP is induced. Expression of pCREB was increased in the right but not left PAG following predator stress in the same area in which right ACE–PAG LTP persists following predator stress (Adamec et al. 2003). Moreover, the same three aspects of the predator stress experiences (one cat behavior: biting, and two rat behaviors: active defense and time immobile) were highly predictive of both pCREB measured 20 min after predator stress, and right ACE–PAG LTP measured 11 or 12 days after predator stress (Adamec et al. 2003), providing a further link between pCREB expression and LTP. The lateralized increase in PAG pCREB expression is also NMDA receptor dependent, in that it may be blocked by 10 mg/kg CPP given 30 min prior to predator stress (Blundell, in preparation).

pCREB expression is also increased in the amygdala 20 min after predator stress. In this discussion we concentrate on BLA and ACE, although other nuclei are af-

fected (see Adamec et al. 2005a). As with the PAG, the same aspects of the stress situation (cat bites, rat active defense, and rat time immobile) were highly predictive of both pCREB in ACE and ACE–PAG LTP. In BLA, rat time immobile was highly predictive of both pCREB in BLA and right VAB–BLA LTP measured 11 or 12 days after predator stress (Adamec et al. 2005a). The NMDA dependence of amygdala pCREB changes has recently been established (Blundell, in preparation).

3.4. Does Predator Stress Engage Learning-Like Mechanisms?

Both the VAB–BLA and ACE–PAG pathways have been implicated in contextual fear conditioning (Carrive et al. 2000; Maren and Fanselow 1995). The predator stress-induced increase in pCREB expression implicates engagement of learning-related neural plasticity (Silva et al. 1998) and possible synaptic restructuring (Adamec et al. 2003). PTSD symptoms have been characterized by some as indelible and terrible memories of the traumatic experience (van der Kolk 1997). Moreover, there is interest in the clinical literature in preclinical research into fear memory establishment and modulation as a source of clues to novel therapies. A notable example is studies of catecholaminergic involvement in memory consolidation. Beta adrenergic receptor blockade (e.g., with propranolol) in animals shortly after contextual fear conditioning blocks consolidation of long-term memory (Cahill et al. 2000; Ji et al. 2003). Similar effects of propranolol on consolidation of emotional memory is found in humans (Cahill and van Stegeren 2003). Finally, poststressor propranolol ameliorates subsequent symptoms of PTSD in humans (Pitman et al. 2002; Vaiva et al. 2003).

Studies of the poststressor effects of propranolol on predator stress are in progress. To date, however, this question of shared learning and stress mechanisms has been approached in two other ways. Engagement of pCREB-related pathways and possible synaptic restructuring implies protein synthesis. One way to test this idea is to suppress protein synthesis after stress to block consolidation of effects. This has been done in both consolidation and reconsolidation paradigms (see below). The second approach was to ask if other gene expression pathways implicated in LTP and memory formation might be engaged by predator stress. One such pathway involves the immediate-early gene, early-growth response 1 gene (*egr-1*, also called *zif268*, *ngfi-a*, *krox 24*, *tis-8*). *Egr-1* mRNA expression increases in the lateral nucleus of the amygdala following contextual fear conditioning (Malkani and Rosen 2000a, b) and is associated with induction of limbic system LTP (Ribeiro et al. 2002).

3.4.1. Consolidation and Reconsolidation of Predator Stress Effects on Behavior

Consolidation is a process of continued neural change after initial acquisition of a

memory. It is inferred by the fact that it may be interrupted after memory acquisition. Consolidation may be blocked by protein synthesis inhibitors, such as anisomycin given systemically (Davis et al. 1980, 1981; Davis and Squire 1984), injected icv (Debiec et al. 2002), or injected directly into brain areas. It is of particular interest that consolidation of long-term memory, but not short-term memory, during Pavlovian fear conditioning is blocked by immediate posttraining injection of anisomycin into the basolateral amygdala (Schafe and LeDoux 2000b). Finally, there is evidence that protein synthesis inhibition with anisomycin blocks induction of LTP in limbic pathways including the hippocampus and amygdala (Barea-Rodriguez et al. 2000; Okulski et al. 2002).

Of further interest is reconsolidation. It has been suggested that memories become labile upon rearousal and require new protein synthesis for their reinforcement. Reexposure of rats to conditioned fear provoking cues increases pCREB expression in basolateral and central amygdala, implicating pCREB in reconsolidation (Hall et al. 2001). Moreover, it is possible to disrupt the original fear memory during rearousal by protein synthesis inhibition (Nader et al. 2000b). Injection of anisomycin into the basolateral amygdala after reexposure of rats to a tone previously paired with a shock interfered with subsequent freezing to the tone tested 24 h later (Nader et al. 2000a). Because reexposure therapy is used to treat PTSD, and if stress-induced changes in affect follow fear learning-like mechanisms, then use of protein synthesis inhibition during such therapeutic sessions might facilitate the undoing of the indelible memories of PTSD.

The involvement of protein synthesis in consolidation and possible reconsolidation of effects on affect of predator stress were therefore investigated. These findings are in press (Adamec et al. 2005c) and only a brief account is given here. One hundred and sixty male Long Evans hooded rats were assigned initially to four groups of 20 within two testing paradigms; consolidation and reconsolidation. The four groups were handled controls, predator stressed (exposed to a cat for 10 min), predator stressed + vehicle, or predator stressed + anisomycin. Handling and cat exposures were as described in Adamec et al. (2003). In the consolidation paradigm, groups were handled or cat-exposed once. In the reconsolidation paradigm, groups were handled or cat-exposed twice, but only injected on the second treatment. Repeated cat exposures were separated by 1 week.

All injections were given 1 min after cat exposure and were subcutaneous in a volume of 1 ml. Anisomycin (210 mg/kg) was suspended in 1 ml of Tween 80 solution (two drops of Tween 80 sonicated in 10 ml of sterile saline). Vehicle was Tween 80 solution. This high dose of anisomycin was used to ensure up to 8 h of nearly 80% suppression of protein synthesis (Davis et al. 1980) to cover a possible second wave of pCREB expression and possible protein synthesis observed in stress (Bilang-Bleuel et al. 2002), LTP (Schulz et al. 1999), and learning paradigms (Stanciu et al. 2001).

One week after treatments involving injection, rat affect was tested in the EPM, light-dark box, social interaction, and acoustic startle tests (following methods in Adamec et al. 2003). Predator stress altered behavior, as seen previously (Adamec et al. 2003) in all tests except in the light-dark box, where it was without effect.

Moreover, there was no evidence that anisomycin blocked consolidation or reconsolidation of effects of predator stress on social interaction, or delay of habituation of startle. The only effects of anisomycin were observed in the consolidation paradigm with effects on startle amplitude and in the EPM.

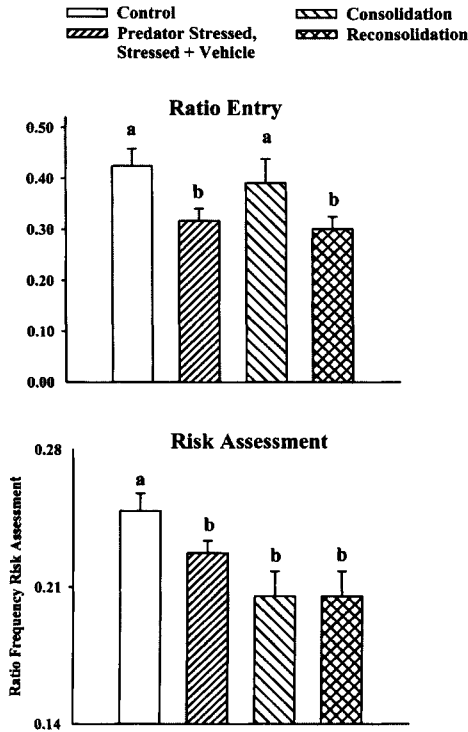


Fig. 2. Plotted in the *upper panel* are mean + SEM square root of open arm exploration in the elevated plus maze given as ratio entry. Ratio entry is a ratio of the number of entries into the open arms to the total number of entries into open and closed arms. Means are from four groups. Control is handled control. Predator stressed, stressed + vehicle groups are combined from those exposed to a cat once or twice with or without an injection of vehicle immediately after exposure, as described in the text. The consolidation group includes rats exposed to a cat once and injected with anisomycin immediately after exposure. The reconsolidation group is rats exposed to a cat twice and injected with anisomycin immediately after the second exposure. Mean + SEM square root of risk assessment of the same four groups is plotted in the *lower panel*. Risk assessment in the plus maze was measured as the frequency of stretch-attend explorations of the open arms with hindquarters in a closed arm as a ratio of time in the closed arms. Within a given panel, means marked with different letters differ from each other. Means marked with the same letter do not differ

Because repeated predator stress with or without vehicle injection did not alter the response to predator stress in the plus maze, consolidation and reconsolidation paradigm handled groups were combined as well as consolidation and reconsolidation, cat-exposed, and cat-exposed + vehicle groups. These combined groups (handled and predator stressed) were compared to consolidation and reconsolidation predator-stressed groups given anisomycin with one-way analysis of variance. There were group effects for two measures of open arm exploration, ratio time and ratio entry, and ratio frequency risk assessment (all $F\{3,156\} \geq 2.90$, $P < 0.04$, square root transformation to meet test assumptions). Predator stress reduced all measures relative to control. Anisomycin given after one cat exposure (consolidation paradigm) returned open arm exploration to control levels but was without effect on the stress reduction of risk assessment (Fig. 2). Anisomycin given after the second cat exposure (reconsolidation paradigm) did not alter predator-stress reductions of these measures (Fig. 2, planned comparison t test, all $t\{156\} \geq 2.25$, $P < 0.03$). Finally Anisomycin after the first, but not second, cat exposure blocked stress induced increases in startle amplitude (Adamec et al. 2005c).

These findings suggest two conclusions. First, consolidation of some but not all affective changes following predator stress share mechanisms in common with fear conditioning in that they are dependent on protein synthesis. Thus, although predator stress effects on open arm exploration and risk assessment are initiated by NMDA receptor-dependent processes (Adamec et al. 1999a, b), they do not share similar mechanisms of consolidation. Moreover, behavioral changes in social interaction, startle habituation, and EPM, which are likely to be mediated by changes in different neural substrates habituation, also seem to require different neural mechanisms of maintenance of those changes. Second, reexposure to the threat (second cat exposure) does not create a lability of affective changes, which may be disrupted by anisomycin. To the extent that predator stress models neural mechanisms of affective change in PTSD, the absence of reconsolidation effects does not support the idea that protein synthesis inhibition might be an efficacious adjunct to reexposure therapy. However, it is possible that the reexposure paradigm used here was too intense. While reexposure therapy strives for realism, it does not recreate the original trauma, and nor do reconsolidation memory experiments recreate the original conditioning paradigm. Rather, the reconsolidation memory experiments provide conditioned cues to the original threat, such as shock. Reconsolidation-like effects still might be achievable if reminders of the predator stress rather than the predator stress itself were employed. Further work seems warranted.

3.4.2. Egr-1 Expression and Predator Stress

The expression of the immediate-early gene, early-growth response 1 gene (*egr-1*), increases in the lateral nucleus of the amygdala following fear conditioning (Malikani and Rosen 2000a, b). This suggests that *egr-1* expression in the amygdala may be a transcriptional switch for learning of fear, activating later gene expression of long-term memory processes. In collaboration with Rosen (Rosen et al. 2005), we

tested whether *egr-1* mRNA expression is also increased during unconditional fear. Three groups were used: handled control, predator stressed (rats were exposed to a cat for 5 min), and a confined control where rats were loosely confined for 5 min to induce immobility after 3 days of confinement adaptation (as described in Adamec et al. 2003). As measured by in situ hybridization, cat exposure increased *egr-1* mRNA in the periventricular nucleus of the hypothalamus compared with both the confined and handled groups, which did not differ, suggesting that cat-exposed rats were stressed. In contrast, *egr-1* was not increased over handled controls by either confinement or predator stress in any nucleus of the amygdala including lateral amygdala. In the context of predator stress increases of pCREB expression in the amygdala, these data support the hypothesis that fear conditioning and predator stress engage different molecular mechanisms in the amygdala.

3.5. Transmitter System Candidates for Poststressor Prophylaxis

The predator stress model may be used to explore neurotransmitter manipulations after stress that might prevent lasting changes in affect. Several systems with prophylactic potential have been identified. These include cholecystokinin (CCK), serotonin (5-HT), and corticosterone.

CCK has anxiogenic properties in animals and humans (Beinfeld 2001; Bradwejn and Koszycki 2001). Its receptors are localized in the CNS, including in the amygdala and PAG (Liu et al. 1994; Luo et al. 1998; Wunderlich et al. 2002). PAG CCK increases defensive and anxiety-like behaviors via CCK₂ receptors (Luo et al. 1998; Netto and Guimaraes 2004). Lasting behavioral effects of predator stress are prevented by systemic CCK₂ receptor block (but not CCK₁) 30 min before or 30 min after predator stress (Adamec et al. 1997). CCK might act through CCK₂ receptors to prolong NMDA-dependent LTP after its initiation (Yasui and Kawasaki 1995). Moreover, CCK at CCK₂ receptors activates (phosphorylates) mitogen-activated protein kinase (MAPK) (Seva et al. 1997). MAPK activation is implicated in protein synthesis and pCREB-dependent amygdala LTP (Huang et al. 2000), and in consolidation of fear conditioning (Schafe et al. 2000). So CCK acting at CCK₂ receptors may be a stress-activated positive modulator of consolidation of limbic circuit neuroplastic change and lasting change in affect. As such, CCK₂ receptors are a putative target for prophylactic intervention.

Serotonin is implicated in PTSD as chronic administration of selective 5-HT uptake inhibitors (SSRIs) ameliorates many of the symptoms of PTSD (Friedman 1997). Recently, we found that giving vilazodone, an SSRI and selective 5-HT_{1A} agonist, 10 min after predator stress, prevented only stress-induced increase in acoustic startle (Adamec et al. 2004a). There are several explanations for these findings (Adamec et al. 2004a), but one is particularly relevant to the present chapter. Serotonin, binding to 5-HT_{1A} receptors in the lateral amygdala, might act to retard neuroplastic changes leading to hyperarousal (startle) as part of an adaptive reaction to inescapable stress. Enhancing this adaptive action pharmacologically might prevent lasting changes in startle. In support of this idea, sustained rises in sero-

tonin levels have been reported in the basolateral amygdala of rats exposed to inescapable, but not escapable stress (shock) (Amat et al. 1998). Moreover, activation of 5-HT_{1A} receptors blocks NMDA-dependent LTP at rodent lateral amygdala synapses (Pollandt et al. 2003). Finally, cannulation of NMDA receptor blockers into the lateral amygdala 30 min prior to predator stress blocks lasting increases in startle amplitude, but has no effect on behavior in the EPM (Adamec et al. 1999b).

Broader prophylactic protection against predator stress effects on affect is provided by selective 5-HT_{2A} antagonism with EMD 281014 [7-(4-(2-(4-fluorophenyl)-ethyl)-piperazine-1-carbonyl)-1H-indole-3-carbonitrile HCl] (Bartoszyk et al. 2003). EMD 281014 given 10 min after predator stress blocks lasting effects of stress on open arm exploration in the EPM and on response to startle (Adamec et al. 2004b).

Possible mechanisms include blockade of a 5-HT_{2A} receptor-mediated prolonging of NMDA-dependent LTP in BLA (Chen et al. 2003), and/or a blockade of 5-HT_{2A} receptor-mediated release of ACTH, corticosterone, and prolactin, as well as activation of CRF cells in the ACE (Van de Kar et al. 2001). In the context of the path analyses discussed above, blocking of LTP in BLA would be expected to interrupt the serial circuit proposed for startle, and to interfere with the parallel circuit controlling open arm exploration. Add to this a reduced activation of CRF cells in the ACE, suppression of both arms of the parallel circuit proposed for control of behavior in the EPM may occur. Suppression of stress hormones is also attractive as a mechanism. Suppression of steroid synthesis with ketoconazole prior to predator stress prevents raised levels of ACTH, corticosterone, and prolactin in response to predator stress and blocks lasting anxiogenic effects of stress in the EPM (Cohen et al. 2000). Moreover, predator stress elevates plasma corticosterone levels acutely (Adamec et al. 1998). Changes in corticosterone levels following predator stress are long lasting (more than 180 min) and levels are highly predicted by the same aspects of the predator stress experience that are predictive of pCREB and limbic LTP following predator stress (Adamec et al. 2005a). Raising plasma levels of corticosterone to those produced by predator stress by systemic injection of corticosterone has no lasting effects on behavior in EPM, but may lastingly increase startle amplitude (Adamec et al. 2005a). These data suggest corticosterone interacts with other systems activated by the stress experience to initiate lasting neurobehavioral changes, in EPM, at least.

4. Conclusion

Predator stress may model aspects of anxiety and hyperarousal associated with PTSD. Brain changes underlying behavioral changes involve in part NMDA receptor-dependent LTP-like changes in hippocampo-amygdala and amygdalo-PAG transmission. NMDA dependent pCREB cascades are also implicated in initiation of limbic LTP. There is a suppressive role for 5-HT_{1A} and a permissive role for 5-HT_{2A} and CCK₂ receptors in some behavioral changes. Poststress agonism and an-

tagonism of 5-HT_{1A} and 5-HT_{2A}, and CCK₂ receptors, respectively, provide protection against some behavioral changes. Serotonin and CCK may act to modulate longevity of limbic neural plasticity. In addition, modulation of corticosteroid response to stress may play a role in the prophylactic action of 5-HT_{2A} receptors. Consolidation of some stress-induced behavioral changes appears to be protein synthesis dependent. However, repeated exposure to the stressor does not render reconsolidation of behavioral changes vulnerable to protein synthesis inhibition. Study of *egr-1* mRNA expression induced by predator stress does not support the idea that mechanisms common to fear conditioning mediate changes in anxiety-like behavior, at least in the amygdala. There may be a role for selective 5-HT and CCK receptor modulation and protein synthesis inhibition in immediate poststress intervention to prevent lasting changes in affect.

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Altered Emotional Behaviors in Mammalian Bombesin Receptor Knockout Mice: Implication for the Molecular Pathogenesis of Stress-Induced Psychiatric Disorders in Humans

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1. Introduction

Neuropeptides are thought to play important roles in the pathogenesis of various psychiatric disorders, including posttraumatic stress disorder (PTSD) (Merali et al. 2002). To elucidate the pathophysiological role of neuropeptides for the disorders in vivo, we have employed behavioral analyses of gene-manipulated mice lacking their receptors. Among various neuropeptides, we have focused on bombesin, because its receptors are highly expressed in the hippocampus and the amygdala. Both regions are known to mediate memory and emotional behaviors.

Bombesin (BN) is a tetradecapeptide originally purified from the skin of the European frog *Bombina orientalis* (Anastasi et al. 1971). Two BN-like peptides, gastrin-releasing peptide (GRP) and neuromedin B (NMB), have been identified in mammalian tissues (McDonald et al. 1979; Minamino et al. 1983). These peptides exert their effects by binding to G-protein coupled receptors on the cell surface; these are the GRP-preferring receptor (GRP-R) and the NMB-preferring receptor (NMB-R). In addition, a third subtype of mammalian bombesin receptor (BRS-3) has been cloned; however, high affinity natural ligand(s) specific to BRS-3 have not yet been identified (Battey and Wada 1991; Fathi et al. 1993). BN-like peptides and their receptors are widely distributed in the mammalian central nervous system and modulate many aspects of behavior such as spontaneous activity and feeding behavior, as well as learning and memory (Flood and Morley 1988; Flynn 1991; Kirkham et al. 1993; Santo-Yamada et al. 2001). We previously produced three strains of BN-like peptide receptor knockout mice using a gene-targeting method (Ohki-

Hamazaki et al. 1997, 1999; Wada et al. 1997). The resulting mice, deficient in GRP-R, NMB-R, and BRS-3, were used in studies designed to clarify and distinguish the functional properties of BN-like peptides in the brain. As a result, we found that the NMB/NMB-R system may work as a risk factor for stress vulnerability and that the GRP/GRP-R system may play some role in learning and memory.

2. NMB and NMB-R

We investigated the effect of restraint stress on the behavior of NMB-R-deficient mice. We first examined the maternal behavior of the mice before and after the stress for 30 min (Yamada et al. 2002a). Near the end of the stress, three pups were laid individually in corners of each home cage. Immediately following the stress treatment, each experimental virgin female mouse was introduced into the vacant corner of the home cage. The essential maternal indices (licking, pup retrieval, grouping, nesting, and crouching) were measured in the initial and final 5-min observation periods during a 35-min test session. As shown in Fig. 1, the overall ma-

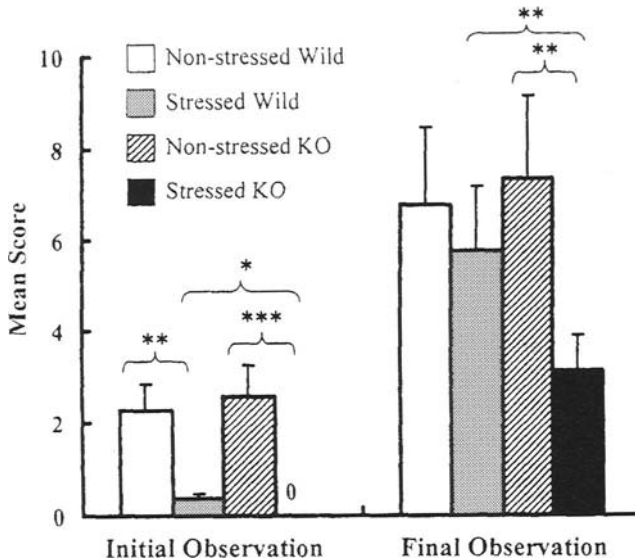


Fig. 1. Overall maternal behavioral performance of neuromedin B-preferring receptor (NMB-R)-deficient mice (*KO*) and wild-type mice with/without 30-min restraint-induced stress. The overall scores represent the sum of the scores of the individual components: pup retrieval, grouping, crouching, and nesting. Data given as mean + quartile deviation. Asterisks represent statistical significance between the indicated groups: *** $P < 0.0001$; ** $P < 0.005$; * $P < 0.03$.

ternal indices at the final observation were significantly lower in stressed NMB-R-deficient mice compared with those of stressed wild-type control mice. At the initial observation, we did not find significant difference of the indices between the two genotypes with stress. The maternal behaviors of wild-type and NMB-R-deficient mice are both suppressed in the initial observations. Furthermore, nonstressed mice show similar indices between the two genotypes. These results suggest that restraint-induced stress can impair maternal behavior in mice, and NMB-R-deficient mice may suffer more severely from the stress than wild-type mice.

Similar vulnerability of NMB-R-deficient mice to the restraint stress was observed in the inhibitory avoidance learning (Yamada et al. 2003). Using a one-trial passive avoidance test, stressed NMB-R-deficient mice exhibited a marked reduction in memory performance (Fig. 2). In the test trial, we did not observe any difference in the mean step-through latency between nonstressed NMB-R-deficient and wild-type mice; however, stressed NMB-R-deficient mice showed significantly shorter latency than stressed wild-type mice and nonstressed NMB-R-deficient mice (Fig. 2). Although NMB-R-deficient mice exhibited elevated spontaneous activity in a novel environment (open field) compared with nonstressed mutant mice after 30 min of stress, a similar difference was also observed between stressed/nonstressed wild-type mice. An elevated plus maze test did not show any effect of the stress stimulus on anxiety in either wild-type or NMB-R-deficient mice. Furthermore, pain response of wild-type and NMB-R-deficient mice induced by electric

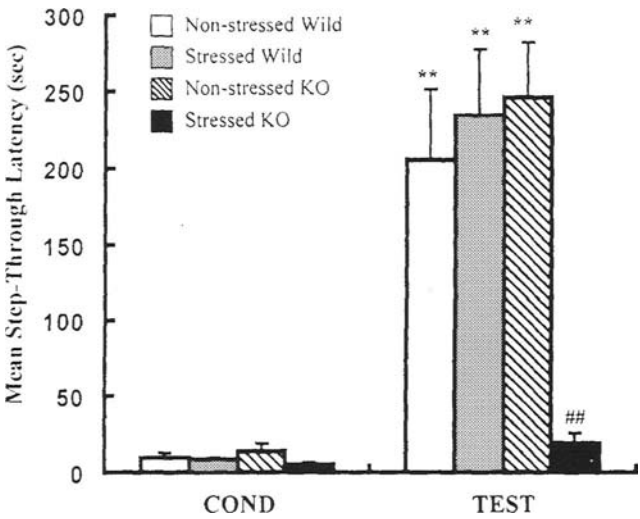


Fig. 2. One-trial passive avoidance test. Data given as mean + SEM. Asterisks indicate statistical significance between the acquisition trial and the test trial ($P < 0.01$); hashes indicate statistical significance between stressed NMB-R-deficient mice and stressed wild-type mice or nonstressed NMB-R-deficient mice in the test trial ($P < 0.01$). COND, acquisition trial; TEST, test trial

footshock was not affected under either stressed or nonstressed conditions. These results indicate that impaired memory performance in stressed NMB-R-deficient mice is not a consequence of changes in spontaneous activity, anxiety, or pain response, and suggest that the NMB/NMB-R pathway plays a role in regulating the stress response via the neural system that controls learning and memory.

We then evaluated the risk assessment behavior of the mice using two representative behavioral paradigms, the light-dark (L-D) box test and the elevated plus maze test (Yamada et al. 2002b). In the L-D box test, there were no significant differences between mutant mice and their wild-type littermates with respect to the conventional parameters such as dark-light (D-L) latency and the duration of staying in the light compartment. However, in the analysis of risk assessment behavior (stretched attend posture), NMB-R-deficient mice exhibited a significant decrease in risk assessment behavior relative to the wild-type cohort (data not shown). Similar to the results of the L-D box test, the analyses of risk assessment behavior from the elevated plus maze test revealed that NMB-R-deficient mice displayed a relative decrease in the frequency of this posture (data not shown). Although risk assessment behavior has yet to gain broad consent as a behavioral index of anxiety, there are reports that this behavior may reflect some emotional state of animals (Yamada et al. 2002b).

Our results suggest that the NMB/NMB-R pathway modulates some forms of emotion (perhaps including anxiety) and constitutes one of the risk factors of stress vulnerability. NMB-R-deficient mice should provide useful information for molecular pathogenesis of stress-induced disorders. Because the NMB/NMB-R system possibly interacts with 5-hydroxytryptamine (5-HT) neurons (Yamada et al. 2002c), further studies should reveal the neural circuits responsible for stress-induced mental disorders including PTSD.

3. GRP and GRP-R

We investigated the role of the GRP/GRP-R system in memory and learning. We first examined the effect of GRP peptide in wild-type mice with drug-induced amnesia. GRP was administered following training in a one-trial passive avoidance test. When scopolamine was used to induce amnesia prior to training, GRP (32 nmol/kg, ip) improved memory performance when the dosage of scopolamine was relatively low (1 mg/kg, ip). We then examined the role of GRP for the acquisition of inhibitory avoidance learning in mice using GRP-R antagonists. An administration of [Leu¹³-(CH₂NH)-Leu¹⁴]BN (antagonizes GRP-R > NMB-R) impaired the performance of inhibitory avoidance learning in all doses (16, 32, 64 nmol/kg). These results suggest that the GRP/GRP-R system plays an important role in memory and learning. Recently, GRP was shown to be important for inhibiting memory specifically related to learned fear (Shumyatsky et al. 2002). We generated specific antibody against the receptor, and found that the antibody is an excellent tool for investigating the expression of GRP-R in the brain (Kamichi et al. 2005). Double-

labeling immunohistochemistry demonstrated that subpopulations of GRP-R are present in GABAergic neurons in the amygdala. Consequently, GRP-R immunoreactivity was observed in the GABAergic neurons of the limbic region. These anatomical results support the idea that the GRP/GRP-R system mediates memory performance by modulating neurotransmitter release in the local GABAergic network.

4. Conclusion

Our results indicate that the NMB-R-deficient mouse is an important tool for investigating the molecular mechanism of stress-induced disorders and developing therapeutic drugs for the disorders. Besides the NMB/NMB-R system, the GRP/GRP-R system is likely to be involved in fear memory. Thus, it is likely that the mammalian bombesin system plays a role in regulating stress response through the neural system that controls learning and memory. Further investigation of the involvement of the mammalian bombesin system in PTSD should provide useful information for the treatment of the disease.

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Role of Stress Hormones and the Amygdala in Creating Lasting Memories

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1. Introduction

Over a century ago, William James asked a critical question that continues to guide current memory research: “Of some [experiences] no memory survives the instance of their passage. Of others, it is confined to a few moments, hours or days. Others, again, leave vestiges that are indestructible, and by means of which they may be recalled as long as life endures. How can we explain these differences?” (James 1890 p 643). Interestingly, Descartes proposed an answer to James’ question over two centuries earlier when he wrote: “The usefulness of all of the passions consists in their strengthening and prolonging in the soul thoughts which are good for it to conserve....” (Descartes 1650). Findings of animal as well as human studies reviewed in this chapter provide extensive evidence supporting Descartes’ hypothesis and have revealed neurobiological processes mediating the “strengthening and prolonging” of memories of significant experiences (McGaugh 2004). Emotionally significant experiences activate hormonal and brain systems that regulate the consolidation of newly acquired memories. These effects are integrated through noradrenergic activation of the basolateral amygdala, which regulates memory consolidation via interactions with many other brain regions involved in consolidating memories of recent experiences (McGaugh 2002, 2004). Thus, the activation of these stress hormone and brain systems enable significant experiences to become well remembered; perhaps as James noted, “...as long as life endures.” Moreover, intense or excessive activation of these systems may, as Pitman (see the chapter by Pitman this volume) has suggested, play a critical role in the etiology of posttraumatic stress disorder (PTSD).

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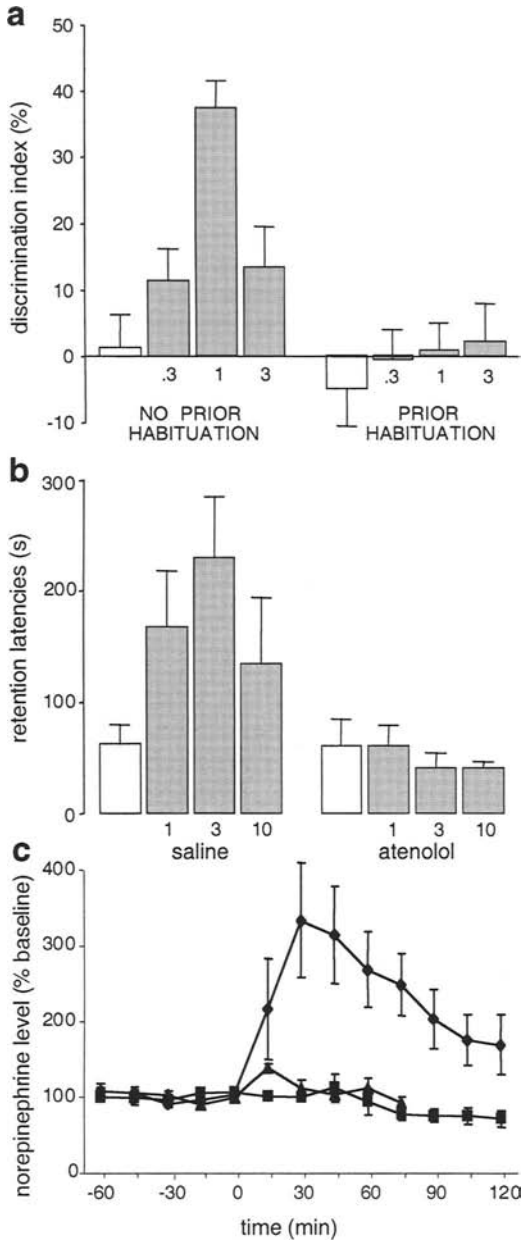


Fig. 1. **a** Corticosterone effects on object recognition memory. Post-training systemic administration of corticosterone (1.0 mg/kg) enhanced 24-h retention in an object recognition task in rats not previously habituated to the experimental context, but did not enhance memory in rats given prior habituation to the context. *Unfilled bars*, vehicle controls; *shaded bars*, corticosterone in ng/kg. From Okuda et al. (2004a). **b** Infusions of the glucocorticoid receptor agonist RU 28362 administered into the basolateral amygdala posttraining enhanced 48-h retention of inhibitory avoidance training. The β -antagonist atenolol (0.5 μ g in 0.2 μ l) infused with RU 28362 blocked the memory enhancement. *Unfilled bars*, vehicle controls; *shaded bars*, RU 28362 in ng. From Roozendaal et al. (2002). **c** Effects of inhibitory avoidance training on norepinephrine levels in the amygdala. Microdialysis probes were inserted into the amygdala via a previously implanted cannula. Samples were collected at 15-min intervals and were automatically injected into a high-performance liquid chromatography system optimized for norepinephrine detection. Rats were given a single footshock during inhibitory avoidance training or in a holding cage. Other rats received no footshock. *Diamonds*, trained rats; *squares*, nonshocked rats; *triangles*, cage-shocked rats. From McIntyre et al. (2002)

2. Stress Hormone Modulation of Memory Consolidation

It is well established that memory can be enhanced by posttraining treatments affecting brain functioning. Findings reported several decades ago indicate that, in rats and mice, posttraining systemic injections of central nervous system (CNS) stimulants enhance long-term memory (McGaugh 1966). Comparable findings have been obtained in experiments with human subjects (Soetens et al. 1993). Such findings provided strong evidence that such posttraining treatments enhance memory by influencing consolidation of recently acquired information (McGaugh 2000) and suggested that learning-induced activation of endogenous systems may play a critical role in modulating memory consolidation. There is now considerable evidence that the adrenal hormones epinephrine (adrenaline) and cortisol (corticosterone, in the rat) released following emotionally arousing experiences modulate memory consolidation (McGaugh and Roozendaal 2002). Early studies in our laboratory (e.g., Gold and van Buskirk 1975) were the first to report that epinephrine administered to rats shortly after training enhanced memory consolidation. Subsequently, many laboratories reported obtaining comparable findings in animal (McGaugh 1983; McGaugh and Gold 1989) and human studies (Cahill 2000; Alkire 2003). There is also extensive evidence that corticosterone modulates memory consolidation in animals (Bohus 1994; de Kloet 1991; Flood et al. 1978; Lupien and McEwen 1997; Roozendaal 2000) and that cortisol enhances memory in human subjects (Abercrombie et al. 2003; Buchanan and Lovaglio 2001). Importantly, such glucocorticoid-induced memory enhancement is mediated by selective activation of low-affinity glucocorticoid receptors (Oitzl and de Kloet 1992). Blockade of glucocorticoid production with the synthesis inhibitor metyrapone impairs memory consolidation (Maheu et al. 2004; Roozendaal et al. 1996a) and prevents stress-induced and epinephrine-induced memory enhancement (Liu et al. 1999; Roozendaal et al. 1996b).

Thus, adrenal hormones enhance the consolidation of memory for emotionally arousing experiences that induce their release. However, whether stress hormones also enhance memories of experiences that are not emotionally arousing is also of interest. We investigated this issue in recent experiments using an object recognition task (Okuda et al. 2004a) that induces a relatively low level of emotional arousal (see Fig. 1a). However, placing rats in a novel testing apparatus does evoke some degree of novelty-induced arousal and habituation of rats to the apparatus is known to reduce this arousal response (de Boer et al. 1990). Corticosterone was administered systemically immediately after object recognition training to rats that were either nonhabituated or well habituated to the experimental context. Corticosterone administered immediately posttraining to nonhabituated rats enhanced their 24-h retention performance. In contrast, corticosterone did not affect 24-h retention of habituated rats given the same training experience (Okuda et al. 2004a). These findings suggest that at least some degree of training-associated emotional arousal is essential for enabling glucocorticoid effects on memory consolidation. To investigate whether training-induced noradrenergic activation is a critical component of

emotional arousal in enabling glucocorticoid effects on memory consolidation in nonhabituated rats, the β -adrenoceptor antagonist propranolol was coadministered with the corticosterone immediately after the object recognition training. As expected on the basis of our findings obtained with other types of emotionally arousing training, propranolol blocked the corticosterone-induced memory enhancement (Okuda et al. 2004b). To investigate whether a pharmacologically induced increase in noradrenergic activity enables glucocorticoid effects on memory consolidation, we administered the α_2 -adrenoceptor antagonist yohimbine to well-habituated rats immediately after object recognition training. Although yohimbine administered alone (in a low dose) did not enhance memory consolidation, corticosterone administered together with yohimbine induced dose-dependent enhancement of memory consolidation (Okuda et al. 2004b).

These findings of animal experiments strongly support the hypothesis that arousal-induced noradrenergic activation is essential in enabling corticosterone enhancement of memory consolidation. Recent human studies have reported evidence consistent with this hypothesis. Buchanan and Lovallo (2001) found that cortisol administered shortly before training enhanced long-term memory of emotionally arousing, but not emotionally neutral, pictures (but see Abercrombie et al. 2003). Human studies investigating the effects on memory consolidation of posttraining administration of epinephrine (Cahill and Alkire 2003) or brief cold pressor stress that induces endogenous stress hormone activation reported similar findings (Cahill et al. 2003).

3. Involvement of the Basolateral Amygdala

Glucocorticoids readily enter the brain and bind to adrenal steroid receptors whereas epinephrine, which does not readily pass the blood-brain barrier, influences memory consolidation by activating peripheral adrenoceptors on the ascending vagus that projects to brain-stem nuclei, including the nucleus of the solitary tract (NTS) (Williams and McGaugh 1993). The NTS sends noradrenergic projections directly, and indirectly via the locus coeruleus, to the amygdaloid complex (Clayton and Williams 2000; Hassert et al. 2004; Ricardo and Koh 1978). Extensive evidence indicates that adrenal hormones released by emotional arousal influence memory consolidation, at least in part, by activating the basolateral amygdala (BLA). The importance of noradrenergic activation of the amygdala in memory consolidation was first suggested by evidence that posttraining intraamygdala infusions of drugs affecting β -adrenoceptors modulate memory consolidation (Gallagher et al. 1981; Liang et al. 1986). Lesions of the amygdala (Cahill and McGaugh 1991) or infusions of adrenoceptor antagonists administered into the amygdala block the memory-enhancing effects of posttraining injections of epinephrine (Liang et al. 1986, 1995). The finding that infusions of β -adrenoceptor agonists administered selectively into the BLA posttraining enhance memory provides additional evidence that the BLA is the critical region of the amygdala involved in the modulation of memo-

ry consolidation (Ferry and McGaugh 1999; Hatfield and McGaugh 1999; LaLumiere et al. 2003).

Glucocorticoid influences on memory consolidation also involve activation of the BLA. Lesions of the BLA, but not of the adjacent central nucleus, block the memory-modulating effects of glucocorticoids (Roozendaal and McGaugh 1996a; Roozendaal et al. 1996c). Moreover, the finding that selective infusions of β -adrenoceptor antagonists into the BLA block glucocorticoid effects on memory consolidation (Quirarte et al. 1997; Roozendaal et al. 2002) indicates that glucocorticoid enhancement of memory consolidation requires concurrent noradrenergic activation (see Fig. 1b). Intraamygdala infusions of β -adrenoceptor antagonists (including propranolol) also block the memory-modulating effects of other neurotransmitter systems, indicating that their effects on memory consolidation are also mediated through noradrenergic activation within the amygdala (LaLumiere et al. 2004; McGaugh et al. 1989).

These findings indicating that activation of adrenoceptors within the amygdala is critical for the modulation of memory consolidation suggest that emotionally arousing learning experiences should induce the release of norepinephrine within the amygdala and that drugs and hormones that enhance memory consolidation should increase the release. Findings of studies using *in vivo* microdialysis and high-performance liquid chromatography to measure norepinephrine levels in the amygdala strongly support these implications. Footshock comparable to that typically used in inhibitory avoidance training significantly enhances norepinephrine levels (Galvez et al. 1996; Quirarte et al. 1998). Moreover, drugs and hormones that enhance memory consolidation potentiate footshock-induced increases in norepinephrine levels in the amygdala (Hatfield et al. 1999; Quirarte et al. 1998; Williams et al. 1998). Additionally, Clayton and Williams (2000) reported that stimulation of the NTS increases norepinephrine levels in the amygdala and enhances memory consolidation.

We also examined increases in norepinephrine levels in the amygdala induced by inhibitory avoidance training (McIntyre et al. 2002). As we expected on the basis of our previous studies (Galvez et al. 1996; Hatfield et al. 1999; Quirarte et al. 1998), norepinephrine levels were increased following training (Fig. 1c). However, the duration of the increased norepinephrine levels was greater than that previously found with footshock stimulation alone. Additionally, and importantly, the amount of increase in norepinephrine levels assessed in individual animals within 90 min after training correlated highly with their subsequent retention performance tested the following day.

4. Involvement of Other Brain Regions in Basolateral Amygdala Influences on Memory Consolidation

The findings summarized above provide strong support for the hypothesis that

arousal-induced noradrenergic activation of the BLA plays a central, and perhaps critical, role in modulating the consolidation of memory for many different kinds of information. The BLA is richly interconnected with many brain regions, including the hippocampus, caudate nucleus, and various cortical regions known to be involved in different aspects or forms of memory. Considerable evidence indicates that the BLA modulates memory consolidation via influences on these other brain regions.

Several experiments in our laboratory have examined BLA influences on the functioning of the hippocampus and caudate nucleus in memory consolidation. In rats trained in a water maze, posttraining infusions of amphetamine into the hippocampus selectively enhanced memory for spatial training, known to involve the hippocampus, whereas amphetamine infused into the caudate nucleus selectively enhanced memory for cued training, a nonspatial task known to involve the caudate nucleus (Packard et al. 1994). In contrast, and consistent with the hypothesis that the amygdala plays a general role in modulating different kinds of memory, amphetamine infused into the amygdala after training enhanced memory for both kinds of training. Other experiments have provided additional evidence that the BLA interacts with the hippocampus in modulating memory for inhibitory avoidance training. In several experiments we found that posttraining intrahippocampal infusions of a glucocorticoid receptor agonist enhance memory and that lesions of the BLA or intra-BLA infusions of a β -adrenoceptor antagonist block the enhancement (Roosendaal and McGaugh 1997; Roosendaal et al. 1999). We also found that the projection from the BLA to the nucleus accumbens, via the stria terminalis, is required for enabling the memory-enhancing effects of glucocorticoids on memory consolidation, including effects involving the hippocampus. Lesions of either the stria terminalis or the nucleus accumbens block the memory enhancement induced by posttraining systemic injections of the synthetic glucocorticoid dexamethasone (Roosendaal and McGaugh 1996b; Setlow et al. 2000) as well as the memory-enhancing effects of a glucocorticoid receptor agonist infused into either the BLA or hippocampus (Roosendaal et al. 2001).

In several recent studies we have also found that the BLA interacts with cortical regions in modulating memory consolidation. As we have found with studies of BLA interactions with other brain regions, an intact BLA is required for modulation of memory consolidation involving the entorhinal cortex, insular cortex, and anterior cingulate cortex (Malin and McGaugh 2003; Miranda and McGaugh 2004; Roesler et al. 2002). The BLA is known to project to the nucleus basalis, which provides muscarinic cholinergic activation of the cortex. Extensive findings indicate that such cholinergic activation is essential for learning-induced cortical plasticity (Weinberger 1998). The evidence that stimulation of the BLA enhances cortical activation and that reversible inactivation of the nucleus basalis blocks BLA-induced cortical activation (Dringenberg and Vanderwolf 1996; Dringenberg et al. 2001) suggests that BLA activation may influence learning and memory consolidation by stimulating cholinergic activation of the cortex. In support of this implication, the findings of a recent experiment in our laboratory indicated that lesions of the nucleus basalis cortical projections blocked the memory-enhancing effects of norepi-

nephriene infused into the BLA posttraining (Power et al. 2002).

In support of our evidence that the BLA regulates memory consolidation via interactions with other brain regions, studies investigating long-term potentiation (LTP) have provided extensive evidence that the BLA influences neuroplasticity in other brain regions, including the hippocampus and cortex. Selective lesions of the BLA or infusions of a β -adrenoceptor blocker into the BLA prevent the induction of LTP in the dentate gyrus (Akirav and Richter-Levin 1999; Frey et al. 2001; Ikegaya et al. 1994, 1997) as well as stress-induced influences on hippocampal and cortical LTP (Kim et al. 2001; Maroun and Richter-Levin 2003). Electrical stimulation of the BLA facilitates the induction of LTP in the cortex and dentate gyrus of the hippocampus (Akirav and Richter-Levin 2002; Dringenberg et al. 2004; Ikegaya et al. 1995; Nakao et al. 2004). Studies also suggest that synchronized oscillatory activity within the BLA may facilitate cortical processes involved in consolidating explicit or declarative memory (Paré 2003; Pelletier and Paré 2004). Pelletier et al. (2005) reported that, in cats, emotional arousal induced by footshock training (inhibitory avoidance) increased both the duration and synchrony of firing of neurons in the

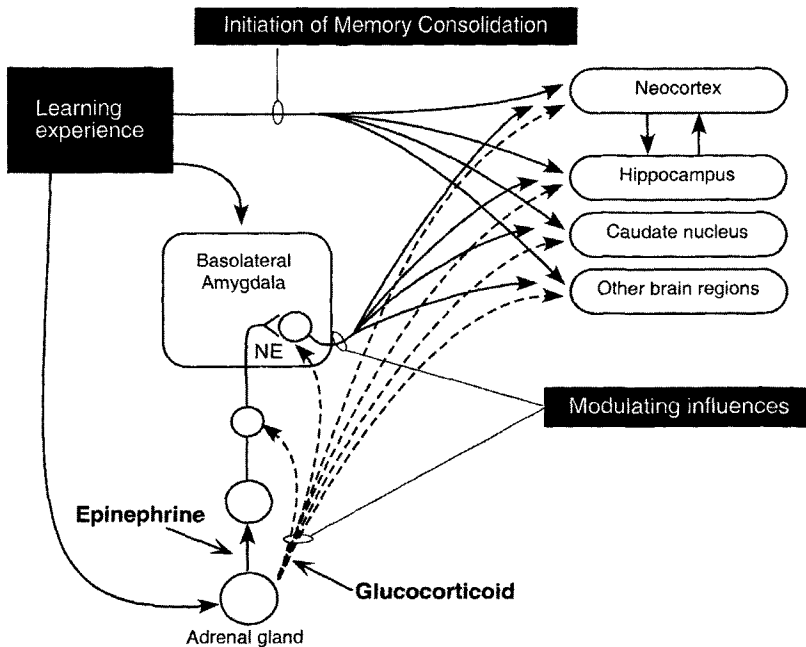


Fig. 2. Emotional arousal-induced modulation of memory consolidation. Experiences initiate memory storage in many brain regions involved in the forms of memory represented. Emotionally arousing experiences also release adrenal epinephrine and glucocorticoids and activate the release of noradrenaline in the basolateral amygdala. The basolateral amygdala modulates memory consolidation by influencing neuroplasticity in other brain regions. From McGaugh (2000)

BLA lasting for 2 h and suggested that the increased firing may serve to promote plasticity in other brain regions.

Figure 2 summarizes schematically the hypothesized roles of adrenal stress hormone and BLA activation in regulating memory consolidation in other brain regions.

5. Emotional Arousal and Lasting Human Memory

The findings of human studies are consistent with those of the animal experiments summarized above in supporting the hypothesis that stress hormones released by emotional experiences influence memory consolidation and that this influence is mediated by activation of the amygdala. Cortisol administered to human subjects prior to presentation of words or pictures enhanced recall tested a day later (Abercrombie et al. 2003; Buchanan and Lovallo 2001). The β -adrenoceptor antagonist propranolol administered to subjects prior to their viewing of an emotionally arousing slide presentation blocked the enhancing effects of emotional arousal on memory (Cahill et al. 1994). Epinephrine or cold pressor stress (known to induce the release of epinephrine and cortisol) administered to subjects after they viewed emotionally arousing pictures enhanced the subjects' long-term memory of the pictures (Cahill and Alkire 2003; Cahill et al. 2003). Similar effects were produced by administration of yohimbine (O'Carroll et al. 1999; Southwick et al. 2002). Schelling et al. (2003) reported that the strength of patient memories of hospital intensive care treatment assessed 6 months after the hospitalization varied directly with the amount of cortisol and epinephrine received during surgery.

Considerable evidence from human studies indicates that the amygdala is involved in the influence of emotional arousal on memory. Memory for emotionally arousing material is not enhanced in subjects with selective bilateral lesions of the amygdala (Adolphs et al. 1997; Cahill et al. 1995). Mori et al. (1999) studied Alzheimer's patients' memories of the 1995 Kobe earthquake 6 months after the quake and also examined the volume of each patient's amygdala using magnetic resonance imaging (MRI). They reported that in patients with different degrees of brain atrophy, the volume of the amygdala correlated highly with the patient memories of their experiences at the time of the quake. Functional brain imaging studies have provided additional evidence that the enhancing effect of emotional arousal on memory involves activation of the amygdala. Cahill et al. (1996) reported that amygdala activity assessed by positron emission tomography (PET) imaging of subjects while viewing emotionally arousing films correlated highly with their recall of the films 3 weeks later. A subsequent study using PET imaging reported that amygdala activity induced by viewing either pleasant or unpleasant slides correlated highly with memory for the slides assessed 1 month later (Hamann et al. 1999, 2002). In a study using functional magnetic resonance imaging (fMRI), Canli et al. (2000) reported that subjects' memory for a series of scenes tested 3 weeks after brain scanning correlated highly with amygdala activity that was induced by view-

ing the scenes; importantly, the relationship between amygdala activity during encoding and memory was greatest for the most emotionally intense scenes.

Other findings of recent brain imaging studies have provided additional evidence consistent with the hypothesis that amygdala activation influences memory processing in other brain regions. A study using “path analysis” modeling of brain activity of subjects assessed (by PET imaging) while they viewed either neutral or emotionally arousing films (Kilpatrick and Cahill 2003) found that emotional arousal increased amygdala influences on the activity of the ipsilateral parahippocampal gyrus. Additionally, Hamann et al. (1999) and Dolcos et al. (2004) reported that the activities in the amygdala and hippocampal/parahippocampal regions are correlated during the encoding of emotionally arousing material.

6. Emotional Arousal and Traumatic Memory

It is, of course, essential for our adaptation and survival that we record and retain lasting memories of our significant experiences. The findings reviewed in this chapter provide strong support for Descartes’ observation that emotional arousal is useful in, “...strengthening and prolonging in the soul thoughts which are good for it to conserve.” However, Descartes also suggested that, “...all the harm that (emotions) can do consists in their strengthening and conserving ... thoughts more than is necessary” (Descartes 1650). Does the activation of stress hormones and the amygdala by emotional arousal strengthen memories “...more than is necessary?” Recent studies have addressed this issue. Delahanty et al. (2005) found that in children who experienced traumatic injuries, elevated levels of epinephrine and cortisol measured immediately after the children were admitted to the hospital correlated with symptoms of PTSD assessed 6 weeks later. Moreover, Pitman et al. (2002, this volume) reported findings indicating that administration of a β -adrenoceptor antagonist to recently traumatized patients can attenuate the development of PTSD. Propranolol administered to patients (for 10 days) within 6 h after they experienced an acute psychologically traumatic event reduced the symptoms of PTSD assessed 3 months after the trauma. Thus, the findings of studies investigating memory of traumatic experiences offer support for Descartes’ hypothesis that emotions can and do strengthen memories more than is necessary. The findings of the experimental studies summarized in this chapter provide some understanding of the neurobiological processes that underlie such strengthening as well as some possible implications for therapeutic intervention to insure that significant events are well remembered, but not excessively remembered.

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Drug Discovery for PTSD: Characterization of an Animal Model of PTSD and PET Imaging of Brain Glucocorticoid Receptor

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1. Introduction

Posttraumatic stress disorder (PTSD) is an anxiety disorder that develops after exposure to life-threatening traumatic experience, characterized by symptoms such as persistent reexperiencing of the traumatic event, avoidance of stimuli associated with the trauma, numbing of general responsiveness, and increased arousal (Pitman 1997). While there is accumulating clinical evidence for appropriate neurochemical and endocrinological hallmarks in patients, there are no effective drugs to treat the disorder. We have tried to develop animal models of PTSD that reflect the neuroendocrinological and behavioral changes seen in patients, with the final goal of identifying novel biological targets for rational drug discovery. Considering the contribution that brain-imaging technologies have made to the understanding of this disorder, we also sought to discover a reliable positron emission tomography (PET) tracer for future clinical use in patients.

2. Characterization of a Rat Model of PTSD

The development of animal models that mimic the symptoms of PTSD patients should provide insights into the pathophysiology of the disorder, and is also vital for discovery of novel therapeutic drugs. Enhanced hypothalamo-pituitary-adrenal (HPA) axis negative feedback is a putative neuroendocrinological hallmark of PTSD

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and can be induced in rats using a trauma-like single prolonged stress (SPS) paradigm (Liberzon et al. 1997). In the SPS model, we first confirmed the enhanced HPA axis suppression by dexamethasone. We next measured behavioral changes induced by the SPS paradigm in rats using two procedures: acoustic startle response and contextual fear conditioning. The acoustic startle amplitude and freezing in contextual fear conditioning were significantly increased in SPS rats when compared with unstressed control rats 7 days after SPS, but not 1 day after SPS, demonstrating gradual alterations with the passage of time. These data suggest that SPS rats exhibit both behavioral (nonhabituation to startle stimuli and enhanced anxiety) and neuroendocrinological (enhanced HPA axis negative feedback) abnormalities seen in PTSD patients.

3. Pharmacological Interventions

Although a number of drugs (e.g., benzodiazepines, antidepressants, adrenergic antagonists) have been tested in clinical trials to explore the possible therapeutic avenues to treat PTSD, no drugs have been consistently proven to be effective. Given the fact that the SPS model reflects at least some of the clinical characteristics of the disease, we sought to discover novel compounds that ameliorate the behavioral deficits in SPS model rats. We first focused on the possible role of brain glucocorticoid receptors (GR) in the development of these behavioral deficits. Rat plasma corticosterone was dramatically increased by SPS, and remained significantly higher than the pre-SPS level 1 day after SPS, but not 7 days after SPS (Fig. 1). To test the possible involvement of GR in mediating these behavioral changes, we exam-

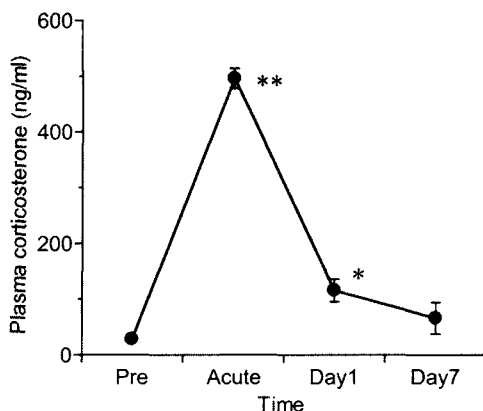


Fig. 1. Alterations of rat plasma corticosterone after single prolonged stress (SPS) ($n = 7$). *Pre*, corticosterone level before SPS, asterisk, $P < 0.05$; double asterisk, $P < 0.01$ vs *Pre* (Dunnett's multiple comparison test). Values are mean $\pm$ SEM

ined the effect of a selective GR antagonist RU40555 (Kim et al. 1998; Pariante et al. 2001) on SPS rats. We confirmed the selectivity of this compound using a GR binding assay (IC_{50} against glucocorticoid receptor: 2.50×10^{-8} M, $K_i = 1.05 \times 10^{-8}$ M, IC_{50} against mineralcorticoid receptor: $>3.20 \times 10^{-5}$ M). RU40555, when administered 5 min before SPS, significantly inhibited SPS-induced enhancement of contextual fear conditioning, suggesting that an activation of GR plays an important role in enhanced anxiety of SPS rats.

4. Gene Profiling Analysis in the SPS Model

To examine the molecular changes underlying the SPS-induced functional deficits, and to discover novel molecular targets for the rational treatment of the disease, we performed comparative microarray analysis using RNA from three brain regions (hippocampus, amygdala, and anterior cingulate cortex) of SPS rats and unstressed controls. One, 28, and 4 genes were differentially expressed in the hippocampus, amygdala, and anterior cingulate cortex, respectively, only at 7 days after SPS, when behavioral abnormalities were observed (Table 1). The largest changes were observed in the amygdala. Among 13 genes upregulated at only 7 days after SPS, 2 genes, the 5-HT_{2C} receptor (Kennett et al. 1997) and the angiotensin I-converting enzyme (Costall et al. 1990) are suggested to be involved in anxiety. These data suggest that these molecules may have the potential for therapeutic targets.

Table 1. Summary for number of genes differentially expressed in hippocampus, amygdala, and anterior cingulate cortex by single prolonged stress (SPS) in rats

Hippocampus			Amygdala			Anterior cingulate cortex		
Day 1	Day 7	Gene number	Day 1	Day 7	Gene number	Day 1	Day 7	Gene number
↑	↑	2	↑	↑	5	↑	↑	0
↑	⇒	0	↑	⇒	6	↑	⇒	3
⇒	↑	1	⇒	↑	13	⇒	↑	1
⇒	↓	0	⇒	↓	15	⇒	↓	3
↓	⇒	0	↓	⇒	0	↓	⇒	1

↑, Upregulated vs unstressed control; ↓, downregulated vs unstressed control, ⇒, no change
Criterion for change: twofold

5. PET Ligand for Glucocorticoid Receptor

The development of a new *in vivo* biotracer for psychiatric disorders, such as PTSD, would be very useful both for clinical diagnosis and for establishing animal disease models. Although imaging by PET for corticosteroid receptors has never been reported, we focused on the development of a new PET tracer for imaging using rats and conscious monkeys. We finally selected RU40555 as a selective GR antagonist, and developed a rapid organic synthesis method for preparation of ^{11}C -labeled RU40555 (Fig. 2), because of the very short half-life of carbon-11 (20.2 min).

We compared two labeling reagents of ^{11}C -RU40555: ^{11}C - CH_3I and ^{11}C - CH_3OTf . As shown in Table 2, a direct *N*-methylation with ^{11}C - CH_3OTf was found to be better than that of ^{11}C - CH_3I . The *N*-methylation with ^{11}C - CH_3OTf at 60°C for 5 min and an injectable solution of ^{11}C -RU40555 was obtained in 22 min from an introduction of ^{11}C - CH_3OTf . The decay-corrected radiochemical yield based on ^{11}C - CH_3OTf was 21.1%, the specific activity was 52 GBq/ μmol , and the radiochemical purity was more than 99%. Whole-body distribution of ^{11}C -RU40555 following intravenous injection was examined using a planar positron imaging system (Takamatsu et al. 2004) in rats, and maximum brain accumulation was observed 20 min after injection as shown in Fig. 3. PET imaging of ^{11}C -RU40555 with conscious rhesus monkeys was also examined using a high-resolution animal PET cam-

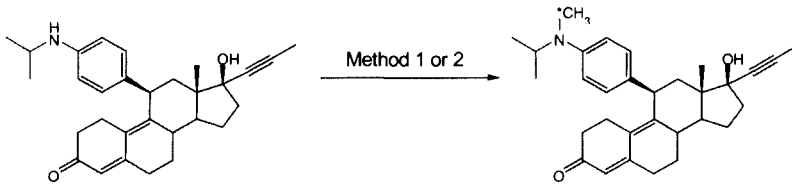


Fig. 2. Chemical structure and the radio labeling route of ^{11}C -RU40555. Asterisk denotes position of ^{11}C labeling. Method 1: ^{11}C - CH_3I , DMI, 150°C , 10 min; Method 2: ^{11}C - CH_3OTf , acetone, 60°C , 5 min

Table 2. Radiolabeled synthetic data of ^{11}C -labeled RU40555

Method	Radio activity (GBq)	Specific activity (GBq/ μmol)	Radiochemical purity (%)	Synthesis time (min)	Yield ^c (%)
1 ^a	0.63	12	>99	32	6.9
2 ^b	2.6	52	>99	22	21.1

^a Method 1: ^{11}C - CH_3I , DMI, 150°C , 10 min

^b Method 2: ^{11}C - CH_3OTf , acetone, 60°C , 5 min

^c The decay-corrected radiochemical yield based on ^{11}C - CH_3I or ^{11}C - CH_3OTf

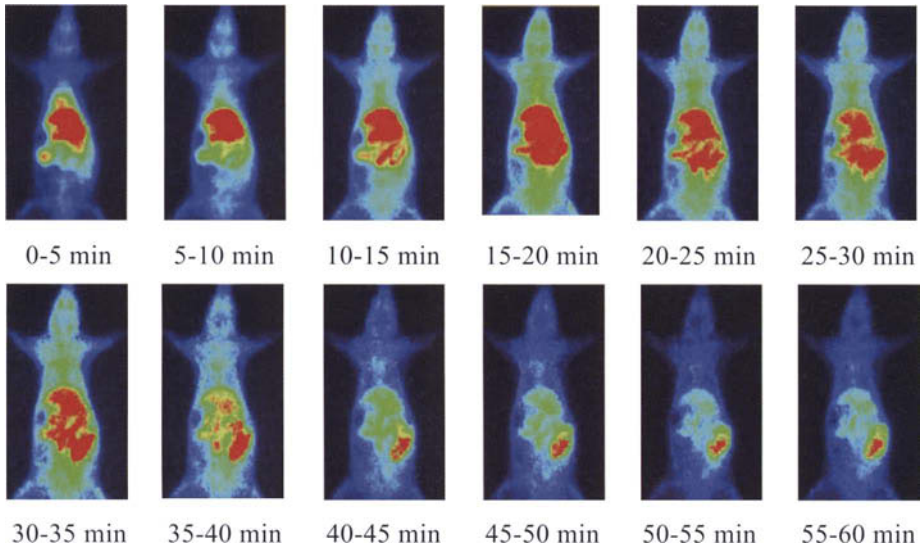


Fig. 3. A typical dynamic series of positron emission tomography (PET) images of whole body distribution of [^{11}C]-RU40555 following intravenous injection ($n = 3$). Each image was accumulated for 5 min

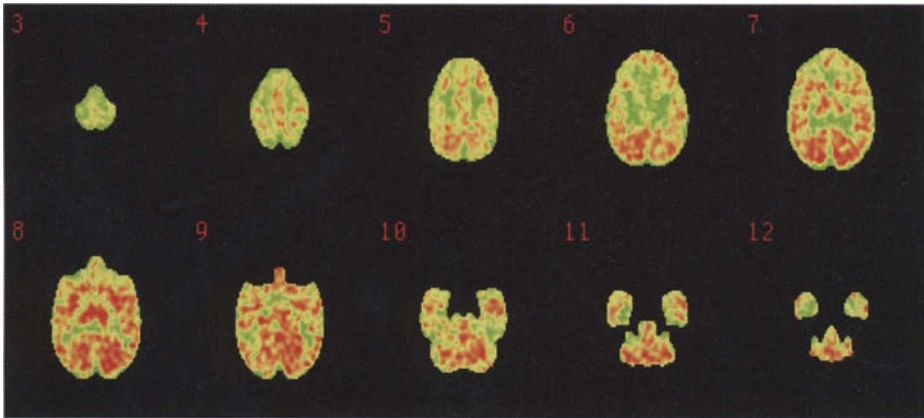


Fig. 4. A typical PET image set accumulated from 30 to 60 min after the injection of [^{11}C]-RU40555. All images were anatomically standardized using NEUROSTAT (Cross et al. 1999) and represent transverse slices at ten levels from the anterior commissure–posterior commissure (AC–PC). The *upper left* image is at AC–PC + 16 mm and the *lower right* image is at AC–PC – 11 mm, with 3 mm between slices

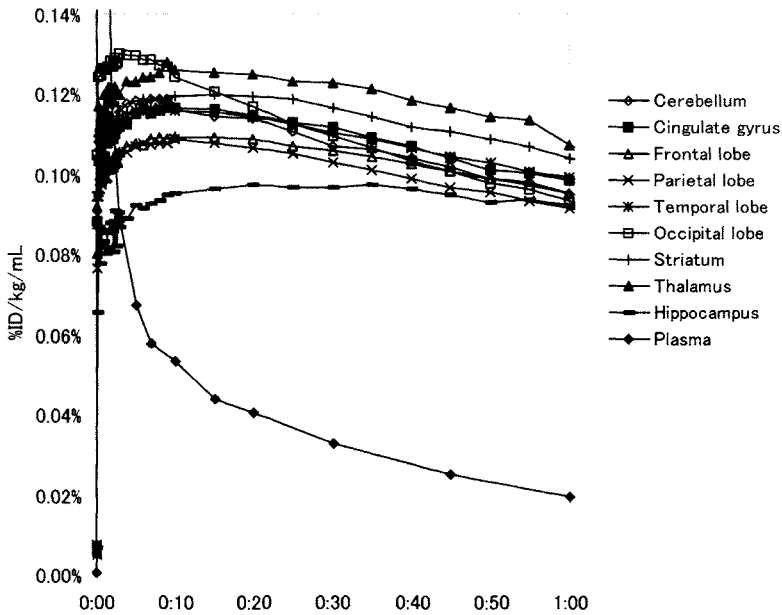


Fig. 5. Time-activity curves of the cerebellum, cingulated gyrus, frontal lobe, parietal lobe, temporal lobe, occipital lobe, striatum, thalamus, hippocampus, and plasma after intravenous injection of [^{11}C]-RU40555 in conscious rhesus monkeys. Each symbol represents the mean of three animals

era (Figs. 4 and 5; SHR7700, Hamamatsu Photonics, Japan). We succeeded in developing a rapid automated synthesis method for [^{11}C]-RU40555, a GR antagonist, which provides a method for measuring GR in the brain.

6. Conclusion

We have confirmed that SPS rats exhibit both behavioral (nonhabituation to startle stimuli and enhanced anxiety) and neuroendocrinological (enhanced HPA axis negative feedback) abnormalities that mimic the symptoms of PTSD patients. Characterization of SPS rats demonstrated that activation of glucocorticoid receptors (GR) is involved in enhanced anxiety, and that several genes may underlie this behavioral change. We also successfully visualized brain GR using PET imaging technology. These findings will accelerate the discovery of putative therapeutic drugs for PTSD, and the imaging of stress-associated signals using PET will provide insight into the pathophysiology of PTSD, as well as aiding the diagnosis and treatment of the disorder.

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Tissue Metabolism of Glucocorticoids: New Controls of Cognitive Function and the Stress Response

Jonathan R. Seckl

1. Introduction

Glucocorticoids (cortisol and corticosterone) have a host of biological effects in brain and body, underpinning adaptive responses to stress and maintaining critical aspects of basal metabolism (de Kloet 2004). Many neural and glial systems are modified by glucocorticoids and their target genes include neurotransmitter systems, receptors, ion channels, cytoskeletal proteins, enzymes, second messenger systems, and metabolism. Acute rises of glucocorticoid levels are adaptive responses to diurnal cues or stress, facilitating survival pathways and inhibiting immediately unhelpful processes such as digestion and inflammation. In contrast, chronic elevations of glucocorticoids (e.g., Cushing's syndrome) are detrimental to homeostasis at all phases of life from embryogenesis to senescence (de Kloet 1991, 2004). In the central nervous system (CNS), chronic glucocorticoid excess exerts profound adverse effects, producing neuropsychiatric dysfunction (depression, psychosis), cognitive impairments, structural deterioration, and neuroendocrine abnormalities (McEwen 1999, 2003). Conversely, chronic severe deficiency of glucocorticoids (Addison's disease) is also deleterious, reducing an organism's survival in stressful circumstances and associating with reduced mood and hippocampal neuronal loss (Sloviter et al. 1989). Thus, it is obviously crucial that glucocorticoid levels are strictly controlled. This is ensured by negative feedback control of the hypothalamic-pituitary-adrenal (HPA) axis. Glucocorticoids act predominantly by binding to intracellular receptors of two types; lower-affinity, widely distributed glucocorticoid receptors (GR) and higher-affinity mineralocorticoid receptors (MR), which have a restricted tissue distribution (de Kloet 1991, 2004). In the hippocampus, where GR and MR are both highly expressed, both receptors bind the same glucocorticoid ligands *in vivo*. Because the genes regulated by GR and MR in the hippocampus are largely distinct (Vreugdenhil and de Kloet 1998), nuclear accessory factors (coactivators and corepressors (Jenkins et al. 2001)) and the fine kinetics of receptor-DNA binding are also likely to be important. However, is the product of

circulating steroid levels and this transcriptional machinery all there is to glucocorticoid biology?

1.1. Enzymes Modulate Tissue Effects of Glucocorticoids

Recently, it has become apparent that prereceptor metabolism of glucocorticoids, notably by 11β -hydroxysteroid dehydrogenases (11β -HSDs), also potently regulates corticosteroid access to receptors. 11β -HSDs, first identified over 50 years ago (Amelung et al. 1953), are microsomal enzymes that catalyse the interconversion of active 11-hydroxy glucocorticoids (cortisol, corticosterone) and their inert 11-keto forms (cortisone, 11-dehydrocorticosterone, 11-DHC), which do not bind to GR or MR. There are two isozymes of 11β -HSD, products of distinct genes. The isozymes differ crucially in tissue distribution and function (Stewart and Krozowski 1999; Seckl 2004a; Seckl and Walker 2004).

11β -HSD type 2, although more recently isolated and cloned (Brown et al. 1993; Albiston et al. 1994), was the first to have its biological function ascribed. 11β -HSD2 is an NAD-dependent high affinity 11α -dehydrogenase. This isozyme is highly expressed in classical aldosterone-selective target tissues (distal nephron, colon) and the placenta, where it acts rapidly to inactivate glucocorticoids to their inert keto forms. 11β -HSD2 action allows selective access of aldosterone (a non-substrate) to intrinsically nonselective MR in vivo in the face of a substantial excess of glucocorticoids in the circulation. Humans (Mune et al. 1995) or mice (Kotelevtsev et al. 1999) homozygous for deleterious mutations of the 11β -HSD2 gene exhibit the syndrome of apparent mineralocorticoid excess in which cortisol and corticosterone occupy intrinsically nonselective renal MR causing sodium retention, severe hypertension, and hypokalaemia. An analogous syndrome is produced by liquorice, the active component glycyrrhetic acid and its hemi-succinate derivative carbenoxolone (CBX), all of which potently inhibit 11β -HSDs (Stewart et al. 1987, 1990). By analogy, any 11β -HSD2 in the CNS might be anticipated to underpin aldosterone selective actions in the brain that cannot be mimicked by glucocorticoids (Seckl 1997).

In contrast, the function of 11β -HSD type 1, although purified and characterized a decade before 11α -HSD2 (Lakshmi and Monder 1988; Agarwal et al. 1989), has been much slower to emerge (Seckl and Walker 2001). It is 11β -HSD1 that is widely expressed in the adult CNS (Moisan et al. 1990a), as well as in liver, adipose tissue, and other peripheral tissues. Although 11β -HSD1 shows bidirectional activity in tissue homogenates and microsomes (Lakshmi and Monder 1988), in intact cells, 11α -HSD1 acts predominantly as a ketoreductase, regenerating active glucocorticoids from inert 11-keto forms. This ketoreductase preference occurs in cells transfected with 11α -HSD1 cDNA (Duperrex et al. 1993; Low et al. 1994a) and in intact primary cultures of hepatocytes (Jamieson et al. 1995), lung cells (Hundertmark et al. 1995), and adipocytes (Bujalska et al. 1997; Napolitano et al. 1998). 11β -reductase activity of 11β -HSD1 also predominates in intact organs and in vivo. Thus, the intact liver perfused in situ converts ~50% of 11-dehydrocorticosterone to active

corticosterone in a single pass, whereas dehydrogenation of corticosterone is four to five times less (Jamieson et al. 2000). In humans, reduction also predominates across the liver (Walker et al. 1992). The subcellular context of 11 β -HSD1 in the inner leaflet of the endoplasmic reticulum (Ozols 1995) is likely to favour 11 β -reduction, because there are potent NADPH-generating enzymes to provide cosubstrate. Indeed 11 β -HSD1 coprecipitates with the NADPH generator hexose-6-phosphate dehydrogenase (Ozols 1995), which may dictate predominant 11-ketoreductase *in vivo* (Draper et al. 2003). Indeed, coinheritance of deleterious mutations in both 11 β -HSD1 and hexose-6-phosphate dehydrogenase may underlie apparent cortisone reductase deficiency in humans. 11 β -reductase regenerates active glucocorticoids in target cells from the substantial levels of inert 11-keto steroids in the blood (Walker et al. 1992) produced by the actions of renal 11 β -HSD2.

Mice homozygous for targeted disruption of the 11 β -HSD1 gene have been generated (Kotelevtsev et al. 1997). These animals show that 11 β -HSD1 is the sole 11 β -reductase. 11 α -HSD1 null mice, despite normal or modestly elevated plasma corticosterone levels, show reduced glucocorticoid action in expressing tissues such as liver and fat and resist the hyperglycemia and dyslipidemia associated with stress or high fat diets (Morton et al. 2001). In contrast, transgenic mice overexpressing 11 β -HSD1 in adipose tissue, at levels chosen to mimic the two to threefold elevation of enzyme activity observed in adipose tissue in human and rodent models of obesity/metabolic syndrome (Seckl et al. 2004a), have elevated intraadipose corticosterone levels, with normal plasma steroid concentrations (Masuzaki et al. 2001). The mice faithfully model the metabolic syndrome and show visceral obesity, hyperglycemia, insulin resistance, dyslipidemia, and hypertension (Masuzaki et al. 2003). Transgenic overexpression of 11 α -HSD1 in liver produces a partial metabolic syndrome phenotype with insulin resistance, hypertension, and dyslipidemia, but without obesity (Paterson et al. 2004). Clearly 11 α -HSD1 potently modulates glucocorticoid action in the periphery. But is this relevant in the CNS and what might it modulate?

2. 11 β -HSDs in the Brain

11 β -HSD activity was first described in the brain of the mouse, rat, dog, and primate in the 1960s (Sholiton et al. 1965; Peterson et al. 1965; Grosser 1966; Grosser and Axelrod 1968; Miyabo et al. 1973). Several studies have shown aldosterone-specific central effects on salt appetite and blood pressure and in some periventricular regions aldosterone is selectively concentrated *in vivo* in the face of corticosterone (Yongue and Roy 1987) and cannot be readily displaced by excess glucocorticoid (McEwen et al. 1986). These data suggest that brain has at least some 11 β -HSD2 activity. However, the presence of 11 β -HSD activity in homogenates of rat cerebellum, which has little or no MR (Moisan et al. 1990b), and in hippocampus (Moisan et al. 1990a; Lakshmi et al. 1991) where the high density of MR are occupied by corticosterone rather than aldosterone *in vivo* (de Kloet et al. 1975) suggest

that 11α -HSD1 is also present. Indeed, it is the 11α -HSD1 isozyme that is widespread in the CNS with high levels in the cerebellum, hippocampus, and neocortex and clearly detectable levels of enzyme in most other brain subregions, including the hypothalamus, amygdala, and brain stem (Moisan et al. 1990a,b; Lakshmi et al. 1991; Sakai et al. 1992; Seckl et al. 1993).

3. 11β -HSD2 in the Adult CNS

Aldosterone, but not corticosterone, mediates central regulation of blood pressure and salt appetite (Gomez-Sanchez 1986; McEwen et al. 1986; Gomez-Sanchez et al. 1990). Aldosterone infused intracerebroventricularly (icv) elevates blood pressure in the rat, whilst the same dose given peripherally is ineffective, demonstrating a specific central action. Corticosterone icv does not reproduce this action and indeed antagonizes aldosterone hypertension (Gomez-Sanchez et al. 1990). Similarly, aldosterone exerts central actions on salt appetite that cannot be reproduced by corticosterone (McEwen et al. 1986). 11β -HSD2 action in the CNS is therefore inferred. This contention is supported by *in vivo* infusion icv of the 11β -HSD inhibitor CBX which increases blood pressure in the rat, an effect reversed by an MR antagonist, suggesting central 11β -HSD2 prevents access of glucocorticoids to central MR (E. P. Gomez-Sanchez and C. E. Gomez-Sanchez 1992). Although 11β -HSD2 mRNA is only found in a few discrete sites in the adult rat brain (Roland et al. 1995; Robson et al. 1998), these loci also express MR and are believed to underlie the selective central effects of aldosterone to increase blood pressure (Gomez-Sanchez 1986) (nucleus tractus solitarius), and modulate salt appetite (Nitabach et al. 1989) (ventromedial hypothalamus, subcommissural organ, central nucleus of the amygdala). In the mouse there is an even more limited distribution of 11β -HSD2, with expression limited to the NTS (Robson and Holmes, unpublished observations). 11α -HSD2 has also not been found in the human (postmortem) CNS, although the brain stem has not been studied (Sandeep et al. 2004). Overall there is very little 11β -HSD2 in the adult CNS.

4. 11β -HSD2 in the Developing CNS

Glucocorticoids are important for normal brain maturation, exerting a range of effects in most regions of the developing CNS (Meaney et al. 1996; Korte 2001; Weinstock 2001; Welberg and Seckl 2001) including the initiation of postmitotic terminal maturation, axodendritic remodeling and the modulation of neonatal brain cell death (Meyer 1983). Prenatal glucocorticoid administration reduces brain weight at birth in sheep (Huang et al. 1999), associated with delays in the cellular maturation of neurons, glia and cerebral vasculature (Huang et al. 2001a), and retarded CNS myelination (Huang et al. 2001b). Given such widespread effects of

glucocorticoids it is not surprising that GR and MR are highly expressed in the developing brain with complex locus-specific ontogenies to permit selective effects (Fuxe et al. 1985; Diaz et al. 1996; Kitraki et al. 1997). However, whether these receptors are occupied by endogenous glucocorticoids until late gestation is not clear, because there is also plentiful 11β -HSD2 in the CNS at midgestation (Brown et al. 1996a; Diaz et al. 1996; Robson et al. 1998). This presumably functions to protect vulnerable developing cells from premature glucocorticoid action. Strikingly, 11β -HSD2 expression is dramatically switched off in a CNS locus-specific manner, mainly at the end of midgestation in the rat and mouse brain. Similarly, in human fetal brain, 11β -HSD2 appears to be silenced between gestational weeks 19 and 26 (Stewart et al. 1994; Brown et al. 1996b). Possibly this widespread gene silencing in the CNS coincides with the terminal stage of brain nucleus development (Brown et al. 1996a; Diaz et al. 1998). Thus, there appears to be an exquisite system of timed protection and then exposure of developing brain regions to circulating glucocorticoids.

The main residual sites of 11β -HSD2 expression in the early postnatal CNS are the thalamus and cerebellum (Robson et al. 1998). The cerebellum still proliferates postnatally and much cell migration, differentiation, and secondary folding takes place between postnatal (P) day 1 and P28. Neonatal adrenalectomy prolongs mitosis and delays disappearance of the external granule layer (EGL), a secondary germinal zone of the developing cerebellum. Exogenous glucocorticoids accelerate EGL disappearance due to premature cessation of granule cell division and inhibition of cell proliferation, decreasing cell numbers within the internal granule layer (IGL) and distorting secondary folding of the lobes (Bohn and Lauder 1978, 1980). The EGL has particularly high expression of 11β -HSD2 (Robson et al. 1998). 11β -HSD2 knockout mice have impaired cerebellar growth (Holmes et al., unpublished), consistent with such a neuroprotective role for 11β -HSD2 in the postnatal cerebellum.

4.1. 11α -HSD2 and Fetal Programming

Human epidemiological studies have revealed that low birth weight at term, presumed to reflect prenatal environmental challenges, associates with an increased later occurrence of cardiovascular (hypertension, ischemic heart disease) and metabolic disorders (type 2 diabetes) in adult life (Barker 1994). In the rat, excessive exposure to glucocorticoids during gestation also lowers birth weight and has life-long effects on the offspring, increasing adult blood pressure, insulin, and glucose levels (Benediktsson et al. 1993; Edwards et al. 1993; Nyirenda et al. 1998; Seckl 2004b). Such effects have been dubbed “glucocorticoid programming” and the brain is a prime target tissue (Matthews 2002; Seckl 2004b). Thus, prenatal administration of dexamethasone, a poor 11β -HSD2 substrate (Albiston et al. 1994; Brown et al. 1996b) that crosses the placenta readily, results in offspring with permanent increases in HPA axis activity (Levitt et al. 1996). This is thought to be the consequence of altered GR and MR expression at sites of glucocorticoid feedback such as

the paraventricular nucleus (PVN), hippocampus, and/or amygdala (Welberg et al. 2001). The precise pattern of GR/MR changes is dependent on the time window of the glucocorticoid exposure and the species involved (Dean and Matthews 1999). In adulthood, prenatal dexamethasone-exposed rats also develop behavioral abnormalities consistent with a more anxious phenotype (Welberg et al. 2001); changes in gene expression in the amygdala may be key. Similar data have been obtained in rats that have been exposed to prenatal stress (Maccari et al. 1995) and also occur following prenatal glucocorticoid exposure in the guinea-pig and sheep (Weinstock 2001; Welberg and Seckl 2001; Matthews 2002; Sloboda et al. 2002). In pregnancy the circadian rhythm of plasma corticosterone is preserved.

4.2. Placental 11 β -HSD2

Whilst lipophilic steroids readily cross the placenta, fetal glucocorticoid levels are much lower than maternal levels (Beitens et al. 1973; Klemcke 1995). This is thought to be due to placental 11 β -HSD2. The enzyme is not a complete barrier to maternal steroids (Benediktsson et al. 1997) and in rodents the peak of the circadian rhythm of plasma corticosterone is able to penetrate the 11 β -HSD2 barrier to some extent (Venihaki et al. 2000). Placental 11 β -HSD2 activity near term shows marked interindividual variation in humans and rats (Benediktsson et al. 1993; Stewart et al. 1995). We hypothesized a decade ago that relative deficiency of placental 11 β -HSD2 leads to overexposure of the fetus to glucocorticoids, retarding fetal growth and programming offspring responses leading to later disease (Edwards et al. 1993). In support of this notion, in rats, lower placental 11 β -HSD2 activity associates with smaller fetuses (Benediktsson et al. 1993). Similar associations have been reported in humans (Stewart et al. 1995; McTernan et al. 2001; Murphy et al. 2002), although not all studies have reproduced this finding (Rogerson et al. 1996, 1997). Markers of fetal exposure to glucocorticoids such as cord blood levels of osteocalcin also correlate with placental 11 β -HSD2 activity (Benediktsson et al. 1995). Rare human cases of 11 β -HSD2 deficiency are described and have very low birth weight (Dave-Sharma et al. 1998), averaging 1.2 kg less than siblings. Although the initial report stated 11 β -HSD2 $^{-/-}$ mice have normal fetal weight in late gestation (Kotelevtsev et al. 1999), this was on a crossed genetic background with necessarily wide confidence intervals. In 11 α -HSD2 $^{-/-}$ mice congenic on the C57Bl/6 background our preliminary data suggest lower birth weight (Holmes et al. 2002).

Inhibition of 11 β -HSD by treatment of pregnant rats with carbenoxolone has effects similar to dexamethasone, leading to offspring of modestly reduced birth weight. This associates with increased passage of maternal corticosterone to the fetal plasma. Although the weight deficit is typically regained by weaning, as with dexamethasone, prenatal carbenoxolone-exposed rats develop adult hypertension (Lindsay et al. 1996a) and hyperglycemia (Lindsay et al. 1996b). These effects of carbenoxolone are independent of changes in maternal blood pressure or electrolytes, but require the presence of maternal glucocorticoids; the offspring of adrenalectomized pregnant rats are protected from carbenoxolone actions upon birth weight or adult physiology.

4.3. *Programming the Hypothalamic-Pituitary-Adrenal (HPA) Axis*

Prenatal 11 β -HSD2 inhibition or bypass with dexamethasone permanently increases basal plasma corticosterone levels in the offspring in rats (Levitt et al. 1996; Welberg et al. 2001). The densities of GR and MR in the hippocampus are reduced in this model, anticipated to attenuate feedback sensitivity. Moreover the glucocorticoid excess may drive, at least in part, the hypertension and hyperglycemia observed in this and other prenatal programming models (Langley-Evans 1997), amplified by increased hepatic and presumably visceral adipose tissue GR (Nyirenda et al. 1998; Cleasby et al. 2003). Similarly in sheep, exposure to betamethasone in utero alters HPA responsiveness in the offspring at up to 1 year of age, although earlier exposure to dexamethasone has no persisting HPA effects in this species (Dodic et al. 2002). Maternal undernutrition in rats (Langley-Evans et al. 1996a) and sheep (Hawkins et al. 2000) also affects adult HPA axis function, suggesting that HPA programming may be a common outcome of prenatal environmental challenge, perhaps acting in part via alterations in placental 11 β -HSD2 activity that is selectively down-regulated by maternal dietary constraint in rats (Langley-Evans et al. 1996b; Bertram et al. 2001) and sheep (McMullen et al. 2004).

4.4. *Programming Behavior*

Overexposure to glucocorticoids in utero, as a result of either prenatal dexamethasone administration or 11 β -HSD inhibition leads to alterations in adult behavior in the rat (Welberg and Seckl 2001). Inhibition of 11 β -HSD, which is most highly expressed in the midgestation brain, produces a phenotype intermediate between early and late gestational dexamethasone exposure (Welberg et al. 2000). Prenatal glucocorticoid exposure also affects the developing dopaminergic system (Diaz et al. 1995, 1997) with clear implications for proposed developmental contributions to schizo-affective, attention-deficit hyperactivity, and extrapyramidal disorders. Indeed, stressful events in the second trimester of human pregnancy associate with an increased incidence of schizophrenia in the offspring (Koenig et al. 2002).

In terms of downstream effects, prenatal dexamethasone or 11 β -HSD inhibition increases corticotropin-releasing hormone (CRH) mRNA levels specifically in the central nucleus of the amygdala, a key locus for the effects of the neuropeptide on the expression of fear and anxiety (Welberg et al. 2000, 2001). Prenatal stress similarly programs increased anxiety-related behaviors along with elevated CRH expression and release in the amygdala (Cratty et al. 1995). The amygdala stimulates the HPA axis via a CRH signal (Feldman and Weidenfeld 1998), which may produce the increased CRH levels in adulthood. A direct relationship between brain GR levels and anxiety behaviors is supported by transgenic mice with selective loss of GR in the forebrain, which exhibit reduced fearfulness (Tronche et al. 1999). Intriguingly, prenatal 11 α -HSD inhibition permanently increases GR and MR in the offspring amygdala (Welberg et al. 2001).

It seems reasonable that fetal programming only occurs at specific critical periods of organ development. Thus, glucocorticoid exposure in the last days of gestation in the rat can target CNS regions actively developing, such as the hippocampus, but not those yet to develop or those already in their final state. The long and complex prenatal and postnatal ontogeny of the brain makes it a prime target for programming (Diaz et al. 1998; Matthews 1998). Whilst the details of brain ontogeny patterns are species specific, the broad impression of tissues protected from or allowing timed exposure to glucocorticoids appears a tenable interpretation of these exquisite patterns of gene expression.

4.5. *CNS Programming Mechanisms*

Indications of the molecular mechanisms by which early life environmental factors program lifespan physiology come from the studies of the processes underpinning postnatal environmental programming of the HPA axis (Levine 1957, 1962; Meaney et al. 1988, 1996;). In these models, short daily handling of rat pups (Meaney et al. 1988) or merely more attentive maternal care to the pups (Liu et al. 1997) during the first 2 weeks of life permanently increases GR density in the hippocampus and prefrontal cortex, but not in other brain regions. This increase in receptor density potentiates the HPA axis sensitivity to glucocorticoid negative feedback and results in lower plasma glucocorticoid levels throughout life, a state compatible with a tighter HPA recovery from environmental stress (Meaney et al. 1989, 1992); note that this may be perceived as advantageous or disadvantageous depending on the actual postnatal challenges experienced. Neonatal glucocorticoid exposure may have similar effects (Catalani et al. 1993).

Such postnatal events act via ascending serotonergic (5HT) pathways from the midbrain raphe nuclei to the hippocampus (Smythe et al. 1994). Activation of 5HT induces GR gene expression in fetal hippocampal neurones in vitro (Mitchell et al. 1990) and in hippocampal neurones in vivo (Yau et al. 1997a). The induction of 5HT requires thyroid hormones, which are elevated by appropriate maternal cues. At the postsynaptic hippocampal neuron, early postnatal events mediate their effects likely via the 5HT₇ receptor subtype, which is regulated by glucocorticoids (Yau et al. 1997b) and positively coupled to cAMP generation (Meaney et al. 2000). The next step appears to involve stimulation of cAMP-associated and other transcription factors, most notably NGFI-A and AP-2 (Meaney et al. 2000). NGFI-A and AP-2 may bind to the GR gene promoter (Encio and Detera-Wadleigh 1991). This pathway might also be involved in some *prenatal* programming paradigms affecting the HPA axis because last trimester dexamethasone exposure increases 5HT transporter expression in the rat brain (Fumagalli et al. 1996; Slotkin et al. 1996), an effect predicted to reduce 5HT availability in the hippocampus and elsewhere. This may well induce a fall of GR and MR, the converse of postnatal higher level maternal care.

The GR promoter is complex, with multiple tissue-specific alternate untranslated first exons in rats (McCormick et al. 2000) and mice (Cole et al. 1995), most within

a transcriptionally active CpG island. All the GR mRNA species give rise to the same receptor protein encoded by exons 2 to 9. Tissue-specific exon 1 usage is regulated by perinatal environment manipulations. Indeed, neonatal handling permanently programs increased expression of only one of the six alternate first exons (exon 1₇) utilized in the hippocampus (McCormick et al. 2000). Exon 1₇ contains sites that may bind AP-2 (Meaney et al. 2000) and have been documented to bind NGFI-A induced by perinatal events (Weaver et al. 2004).

The next crucial issue is how discrete perinatal events can permanently alter gene expression. Evidence has recently emerged for selective methylation/demethylation of specific CpG dinucleotides of the GR gene, notably around the putative NGFI-A site of exon 1₇. These sites are subject to differential and permanent demethylation in association with the level of maternal care; thus, transcription from this promoter is reduced with lower density maternal care (Weaver et al. 2004). Such changes affect NGFI-A binding to the promoter. Indeed, GR under some circumstances can mediate differential demethylation of target gene promoters, which persists after steroid withdrawal, at least in liver-derived cells (Thomassin et al. 2001). This important novel mechanism of permanent determination of the set point of gene control by early life environmental events persisting throughout the lifespan remains to be confirmed in other systems.

4.6. Glucocorticoid Programming in Humans

Glucocorticoid treatment during pregnancy reduces birth weight (Seckl 2004b), but there is a lack of long-term follow-up studies addressing later effects of prenatal glucocorticoid exposure. Antenatal glucocorticoid administration has been linked with higher blood pressure in adolescence (Doyle et al. 2000). A number of studies aimed at establishing the long-term neurological and developmental effects of antenatal glucocorticoid exposure have been complicated by the fact that most of the children studied were born before term and were therefore already at risk of delayed neurological development. In a group of 6-year-old children, antenatal glucocorticoid exposure was associated with subtle effects on neurological function, including reduced visual closure and visual memory (MacArthur et al. 1982). Children exposed to dexamethasone in early pregnancy because they were at risk of congenital adrenal hyperplasia, and who were born at term, showed increased emotionality, unsociability, avoidance, and behavioral problems (Trautman et al. 1995). These effects were seen in genetically unaffected glucocorticoid-exposed offspring. Furthermore, a recent study has shown that multiple doses of antenatal glucocorticoids given to women at risk of preterm delivery were associated with reduced head circumference in the offspring (French et al. 1999). There were also significant effects on behavior; three or more courses of glucocorticoids were associated with an increased risk of externalizing behavior problems, distractibility, and inattention (French et al. 1998). Finally, a recent controlled trial of postnatal dexamethasone in premature babies showed that the steroid was associated with lower subsequent IQ and decrements of other cortical functions (Yeh et al. 2004).

As in other mammals, the human HPA axis appears to be programmed by the early life environment. Higher plasma and urinary glucocorticoid levels are found in children and adults who were of lower birth weight (Clark et al. 1996; Phillips et al. 1998). This appears to occur in disparate populations (Phillips et al. 2000) and may precede overt adult disease (Levitt et al. 2000), at least in a socially disadvantaged South African population. Additionally, adult HPA responses to ACTH stimulation are exaggerated in those of low birth weight (Levitt et al. 2000; Reynolds et al. 2001), reflecting the stress axis biology elucidated in animal models. The HPA axis activation is associated with higher blood pressure, insulin resistance, glucose intolerance, and hyperlipidemia (Reynolds et al. 2001). Finally, the human GR gene promoter has multiple alternate untranslated first exons (Reynolds and Chapman, unpublished observations), analogous to those found in the rat and mouse. Whether these are subject to early life regulation and the molecular mechanisms by which this occurs remain to be determined.

5. 11 β -HSD1 in the Brain

11 β -HSD1-like immunoreactivity and mRNA is widespread in the brain and the anterior pituitary (Moisan et al. 1990a,b; Sakai et al. 1990, 1992). Primary cultures of fetal rat hippocampal cells express 11 β -HSD1, but no 11 β -HSD2 (Rajan et al. 1996). Intact hippocampal cells show only 11 β -reductase activity (Rajan et al. 1996). Licorice derivatives given to rats alter hypothalamic secretagogues in pituitary portal blood in vivo (Seckl et al. 1993) and activate a series of regions of the CNS, including the hippocampus on metabolic mapping studies (Seckl et al. 1991), but does this matter?

5.1. 11 β -HSD1 and HPA Axis Regulation

11 β -HSD1 is expressed in the hippocampus, PVN, and pituitary, which are key loci of glucocorticoid negative feedback. 11 β -HSD1 $^{-/-}$ mice have hypertrophied adrenals and elevated basal ACTH and corticosterone levels at the nadir of the rhythm (Kotelevtsev et al. 1997; Harris et al. 2001) and 11 β -HSD1 $^{-/-}$ adrenals are hyper-responsive to ACTH. These effects are compatible with the increased metabolic clearance of corticosterone in this model due to lack of regeneration in the liver and other sites. However, the unexpected finding is that basal plasma corticosterone levels are also elevated. This would not be expected unless there was altered central drive to or feedback upon the HPA axis, which should otherwise act to maintain normal basal levels of glucocorticoids. Altered central drive is rather unlikely because CRF mRNA levels in the PVN are unaltered. Moreover this is unlikely to be due to altered plasma binding because corticosteroid-binding globulin is not affected by 11 β -HSD1 knockout (Harris et al. 2001). Some data suggest that 11 β -HSD1 $^{-/-}$ mice have attenuated negative feedback because they show less suppression of

corticosterone to exogenous cortisol, 2 h prior to restraint stress. This subtle feedback deficit might explain the elevated basal levels of glucocorticoids in the null mice (Kotelevtsev et al. 1997; Harris et al. 2001). Interestingly, recent data suggest that 11β -HSD1 mRNA expression is reduced in a subset of hippocampal cells in obese Zucker rats (Mattsson et al. 2003). This may in part explain why this strain lacks full HPA feedback sensitivity and hence has increased corticosterone levels.

The circadian rhythm of plasma corticosterone is also modified in 11β -HSD1-null mice. The evening elevation in corticosterone is shifted much earlier, producing an extended period of hypersecretion. 11β -HSD1 activity is not thought to be regulated in a circadian pattern, as 11β -HSD1 mRNA expression is unaltered at 8 a.m. versus 8 p.m. (Harris et al. 2001). The mechanisms and loci of these interesting effects remain to be determined. For all such effects, the role of peripheral metabolism has yet to be reported and merits documentation.

5.2. Regulation of 11β -HSD1 in the CNS

Given the importance of 11β -HSD in determining glucocorticoid action, many studies have addressed the regulation of enzyme activity. Dexamethasone induces 11β -HSD1 gene expression and activity in primary hippocampal cells in vitro and in rat hippocampus, cortex, cerebellum, and hypothalamus in vivo (Low et al. 1994b; Rajan et al. 1996). However, the longer term regulation of 11β -HSD1 by adrenal steroids is more complex (Jamieson et al. 1999). In the tree shrew, chronic psychosocial stress attenuates hippocampal 11β -HSD activity (Jamieson et al. 1997). It is conceivable that the rise in 11β -HSD1 activity in response to some acute stressors may amplify the glucocorticoid signal during its acute adaptive phase, whereas the later down regulation of the enzyme with longer-term stress ameliorates the adverse effects of chronic glucocorticoid excess.

A physiological cause of altered brain 11β -HSD1 has also been reported. In rat pregnancy, the HPA axis becomes rather refractory to stressful stimulation. Interestingly, 11β -HSD1 activity is selectively increased in the hypothalamic PVN at this time (Johnstone et al. 2000) providing the potential to further amplify glucocorticoid feedback and hence flatten HPA responses.

5.3. 11β -HSD1 and Brain Aging

Although the hippocampus requires glucocorticoids for neuronal function and survival, it is also particularly vulnerable to the adverse effects of chronic glucocorticoid excess, which produces atrophy of dendrites, and neuronal and cognitive dysfunction (McEwen 1999). The damaging effects of chronic glucocorticoid excess on neuronal structure and function become more marked with aging. Indeed chronic glucocorticoid overexposure has been implicated in the pathogenesis of age-related cognitive decline and Alzheimer's disease (Meaney et al. 1995). In a subgroup of aged rodents, there is an association of cognitive decline with a chronic elevation of

plasma corticosterone levels. If glucocorticoids are maintained at low levels, by adrenalectomy and low dose corticosterone replacement, age-related impairments are reduced (Landfield et al. 1978). So might 11 β -HSD1 contribute to the wear and tear effects of aging?

In primary cultures of hippocampal cells, 11 β -HSD1 potentiates kainic acid-induced neurotoxicity not only by corticosterone, but equally by intrinsically inert 11-dehydrocorticosterone, an effect prevented by carbenoxolone (Rajan et al. 1996). 11-Dehydrocorticosterone in vivo also increases kainic acid toxicity in adrenalectomized rats, an action also blocked by carbenoxolone (Ajilore and Sapolsky 1999). However, the balance of effects of carbenoxolone, which inhibits both 11 β -HSDs and gains rather patchy tissue access, is difficult to predict.

Old (24 months) wild-type mice, as aged rats, show glucocorticoid-associated impairments in hippocampus-dependent learning and memory tasks in the water maze (Yau et al. 2001). Young 11 β -HSD1 $^{-/-}$ mice, despite elevated plasma corticosterone levels, perform as well as young wild type, suggesting that their brains are relatively immune to their modestly elevated plasma glucocorticoids. Indeed, aged 11 β -HSD1 null mice learn as well as young mice and avoid the cognitive decline seen in the majority of aged wild type mice. This cognitive protection in aged 11 β -HSD1 null mice associates with substantially *reduced* intrahippocampal corticosterone levels, indicating the potency of intracellular metabolism by 11 β -HSDs in determining effective glucocorticoid action upon target receptors. Very recently, selective 11 β -HSD1 inhibitors have been found to reduce blood glucose levels in hyperglycemic mice (Alberts et al. 2002; Barf et al. 2002). Whether these compounds also produce the beneficial cognitive effects seen in 11 β -HSD1 $^{-/-}$ mice remains to be reported.

5.4. 11 β -HSD1 in the Human Brain

In situ hybridization studies in postmortem human brain material have shown high expression of 11 β -HSD1 mRNA in the hippocampus, prefrontal cortex, and the cerebellum (Sandeep et al. 2004), mirroring the findings in rodents. The mRNA is paralleled by 11 β -HSD1 activity. In two small, randomized, double-blind, placebo-controlled, crossover studies, administration of the 11 β -HSD inhibitor carbenoxolone (100 mg 3 times/day) improved verbal fluency after 4 weeks in 10 healthy elderly men (aged 55–75 years) and improved verbal memory after 6 weeks in 12 patients with type 2 diabetes (52–70 years). Although carbenoxolone has been reported to enhance hepatic insulin sensitivity (Walker et al. 1995; Andrews et al. 2003), there were no changes in glycemic control or serum lipids to explain the results. Plasma cortisol was unaltered, suggesting that 11 β -HSD1 does not modulate HPA axis feedback in humans, at least at the morning peak. Intriguingly, 11 β -HSD1 may play a role in pathogenesis, as haplotypes of the HSD11B1 gene associate with a risk of Alzheimer's disease (de Quervain et al. 2004).

6. 5 α -Reductase Type 1

A body of literature documents the role of 5 α -reductase type 1 (and type 2, the isozyme predominating in sexually dimorphic tissues) in determining androgen action in the CNS and periphery (Thigpen et al. 1993). However, its role in glucocorticoid metabolism has been little studied. 5 α -Reductase catalyzes the irreversible reduction of the 4,5 double bond in the glucocorticoid A-ring to form 5 α -dihydrocortisol or 5 α -dihydrocorticosterone, which are rapidly further metabolized by widespread 3 α -hydroxysteroid dehydrogenases to produce polar tetrahydro metabolites that are readily excreted. 5 α -Reductase type 1 is expressed in liver, nongenital skin, the CNS, and adipose tissue. In congenital and acquired syndromes of 11 β -HSD deficiency, there are also changes in the relative excretion of 5 α -tetrahydrocortisol and 5 β -tetrahydrocortisol (Phillipou et al. 1996; A. Jamieson et al. 1999), suggesting a complex interplay between the different pathways of cortisol metabolism. 5 α -Reductase type 1 is apparently regulated by insulin, lipids, and substrate availability (Torres and Ortega 2003). Mice with transgenic deletion of 5 α -reductase type 1 have been generated (Mahendroo et al. 1997). They have no major phenotypic abnormality, although some behavioral effects have been noted (Frye et al. 2004). However, glucocorticoid biology in CNS and periphery has not been reported in these models. Human congenital 5 α -reductase type 1 deficiency has not been described.

So why might this be relevant in a text on CNS effects of glucocorticoids? The peripheral metabolic clearance rate of active glucocorticoids will contribute to plasma levels of cortisol and/or corticosterone and thus affect feedback regulation of the HPA axis. In circumstances of impaired peripheral inactivation of cortisol (e.g., 11 β -HSD2 deficiency (Ulick et al. 1979) or, presumably, hepatic 5 α -reductase deficiency), total cortisol production rate falls in order to maintain normal plasma cortisol levels. In circumstances of enhanced cortisol clearance, e.g., 11 β -HSD1 deficiency (Phillipou et al. 1996; A. Jamieson et al. 1999) or, in theory, increased hepatic 5 α -reductase, plasma cortisol tends to fall, but this is corrected by enhanced ACTH-dependent cortisol secretion. This would lead to adrenal hyperresponsivity to stress and other stimuli. However, a recent complication to this notion of 5 α -reductase and HPA function is the demonstration that 5 α -dihydro- and 5 α -tetrahydroglucocorticoids bind and activate GR *in vitro* and suppress ACTH *in vivo* (McInnes et al. 2004). Thus if 5 α -reductase type 1 is decreased, as may occur with early life events (Arai and Gorski 1968; Reznikov et al. 2004) that may predispose to neuropsychiatric pathologies, the balance between increased glucocorticoid production and the loss of GR agonist metabolites remains uncertain. The role of these enzyme systems in early life events that predispose to PTSD and other disorders and indeed in adults with the disorders is therefore under active investigation.

7. Conclusion

11 β -HSDs are microsomal enzymes that interconvert active glucocorticoids and inert 11-keto forms. This otherwise arcane reaction is surprisingly important in peripheral tissues and the CNS, determining steroid access to nuclear receptors and hence biological effects in health and disease. 11 α -HSD1 is a ketoreductase that amplifies intracellular glucocorticoid levels. Studies in 11 α -HSD1 null mice show a role in feedback. Deficiency of this isozyme reduces intracerebral glucocorticoid levels and prevents the emergence of cognitive deficits with aging. 11 α -HSD2 is a potent 11 α -dehydrogenase, little expressed in the adult CNS, but which plays an emerging role in gating glucocorticoid actions on the developing nervous system. The maintenance of feto-placental 11 α -HSD2 and the inhibition of 11 α -HSD1 have therapeutic potential.

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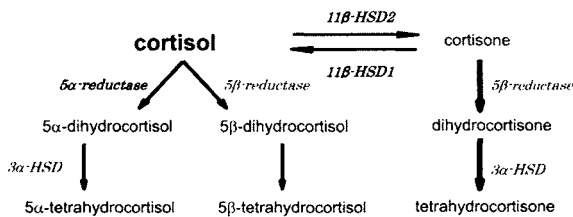


Fig. 1. The major pathways of glucocorticoid metabolism in humans (for mice and rats cortisol is replaced by corticosterone)

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Maternal Deprivation in Neonatal Period and Biological Rhythms

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1. Introduction

Maternal deprivation in the neonatal period is known to induce a variety of endocrinological and behavioral changes in grown-up rats (Weaver et al. 2004). In particular, the responsiveness of the hypothalamus-pituitary-adrenocortical (HPA) axis to noxious stimuli is repeatedly reported to change in adulthood. Maternal deprivation is also known to reset the circadian clock of neonatal rats in the absence of light (Viswanathan and Chandrashekar 1985). The circadian clock located in the suprachiasmatic nucleus (SCN) entrains to light–dark cycles through the retinohypothalamic tract as early as postnatal day 6 (Davis and Reppert 2001). Until this developmental stage, the circadian clock is entrained by the periodicity of their pregnant and nursing mothers, which is called maternal entrainment (Ohta et al. 2003). Separation of newborn pups from their nursing mother for 12 h from postnatal day 1 to day 6 phase-reversed the circadian rhythms in clock gene expression in the SCN in rats deprived of light.

The mechanisms of the long-term effect of maternal deprivation are not well understood. Expressed symptoms are likely triggered by a lack of specific conditions associated with maternal nursing, which include tactile, olfactory, thermal, nutritional, and auditory stimuli (Kuhn and Schanberg 1998). In order to have better insights into the mechanism of maternal deprivation on the circadian clock, we examined certain aspects of maternal deprivation that may contribute to the long-term effect, such as the length of deprivation in a day, the time of day of deprivation, and the ambient temperature during deprivation. We assessed the body weight gain, the stress-induced and basal levels of plasma corticosterone in adulthood, and the circadian phases and period of locomotor activity rhythm at weaning. We further examined the so-called critical periods for the change in these functions.

2. Effects of Maternal Deprivation on the Circadian Parameters of Locomotor Activity Rhythm

Rat pups were blinded immediately after birth and divided into eight groups: MD12 in which maternal deprivation was performed for 12 h in the light period of LD 12:12, for 6 h in the morning (MD6am), for 6 h in the afternoon (MD6pm), for 3 h in the early morning (MD3Eam), for 3 h in the late morning (MD3Lam), for 3 h in the early afternoon (MD3Epm), for 3 h in the late afternoon (MD3Lpm), and the control group in which pups were nursed throughout a day (Yamazaki et al. 2005a). Maternal deprivation was carried out from postnatal day 1 to day 6. Pups were weaned at postnatal day 21 and transferred to individual cages. Spontaneous locomotor activity was continuously monitored with a thermal sensor for 4–5 weeks. The two circadian phases, the activity onset and end of activity, on the day of weaning were estimated by extrapolated regression lines, and the free-running period was calculated.

Table 1 demonstrates the circadian phases at weaning and the free-running periods of locomotor activity rhythm of pups subjected to maternal deprivation under different conditions. The body weights of pups are also demonstrated. The circadian phases were significantly shifted by maternal deprivation, but the extent of phase-shift depended on the length as well as the time of day of maternal deprivation. The circadian phases in MD12 and MD6 were almost reversed, regardless of whether it was imposed in the morning or in the afternoon. On the other hand, a clear effect of time of day was observed when maternal deprivation was performed for 3 h. It was effective when carried out in the morning but had no effect in the afternoon. The free-running period was not significantly affected by maternal deprivation. The body weights of pups in maternal deprivation of longer periods decreased significantly, but were not necessarily related with the amount of phase-shift induced by

Table 1. Body weights, circadian phases and free-running periods of locomotor activity rhythm at weaning of blind pups subjected to maternal deprivation of different conditions

MD condition	Body weight ^a (g)		Circadian rhythm		
	P7	P21	Activity onset ^b (P21)	Activity end ^b (P21)	Free-run period ^c (P21–P31)
MD12	12.63 ± 0.20**	43.07 ± 0.59**	11:48 ± 51**	0:15 ± 12**	24.44 ± 0.06
MD6am	14.85 ± 0.42*	45.13 ± 0.42*	8:32 ± 44**	20:12 ± 52**	24.45 ± 0.05
MD6pm	13.89 ± 0.54*	46.64 ± 1.59	11:47 ± 50**	22:52 ± 63**	24.34 ± 0.04
MD3Eam	18.23 ± 0.43	52.21 ± 0.74	3:50 ± 18*	15:47 ± 13*	24.42 ± 0.05
MD3Lam	17.28 ± 0.48	53.31 ± 0.85	7:40 ± 45*	19:12 ± 37*	24.49 ± 0.04
MD3Epm	16.51 ± 0.84	52.77 ± 1.67	23:10 ± 58	10:40 ± 35	24.47 ± 0.04
MD3Lpm	18.65 ± 0.68	52.47 ± 1.04	22:57 ± 44	10:17 ± 70	24.45 ± 0.05
Control	17.21 ± 0.52	50.36 ± 0.81	0:22 ± 82	11:42 ± 73	24.46 ± 0.05

^a Expressed as mean ± SE (*n* = 5 or 6)

^b Expressed as mean (h:min) ± SE (min)

^c Expressed as mean (h) ± SE

* *P* < 0.05, ** *P* < 0.01 statistically significant difference from control

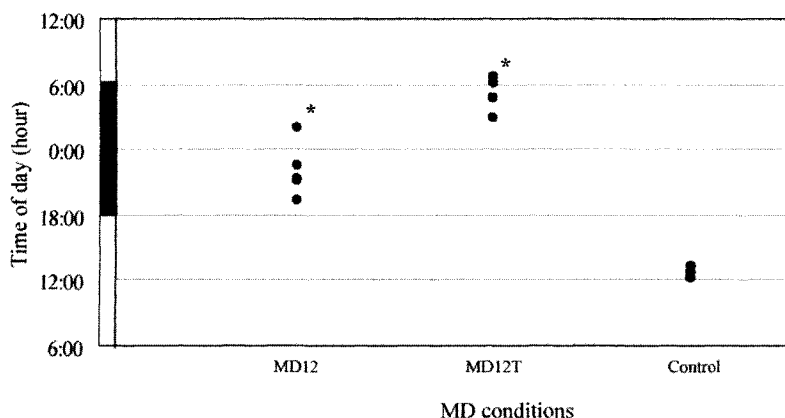


Fig. 1. The activity ends of circadian locomotor rhythms at weaning in MD12, MD12T and the control. *Closed circles* in each group indicate the circadian phases of individual pups. *Asterisks*, statistically significant difference when compared with the control; $P < 0.01$

maternal deprivation.

The findings are consistent with the idea that maternal deprivation resets the circadian clock as a time cue, because the effect was either dependent on the time of day and the strength (length) of maternal deprivation just as in the case of light. However, it is not known what real time cue is associated with maternal deprivation. It might be nursing behaviors of the mother rat, milk supply, a certain substance in the milk, or absence of the mother. In the circadian system of other organisms, ambient temperature is known to act as a time cue (Daan and Aschoff 2001). Therefore, it is interesting to test whether ambient temperature during maternal deprivation might influence the phase-shifting effect of maternal deprivation.

Figure 1 illustrates the activity onsets at weaning in individual pups that were subjected to maternal deprivation for 12 h but kept at an ambient temperature of 37°C with 70%–80% relative humidity. The circadian phase was significantly affected, suggesting that ambient temperature acts at least partly as a time cue in maternal entrainment.

The responsible time cue associated with maternal deprivation is not known. Because the frequency and intensity of maternal care have daily rhythms and are predominant in the early light phase (Lee and Williams 1977), it is not unreasonable to surmise that maternal care is the time cue. However, the effects of cross-fostering by nursing mothers with phase-reversed circadian rhythms were less potent than maternal deprivation (Ohta et al. 2002). Thus, the absence of the nursing mother rather than the presence seems to be a more potent time cue. In this respect, it is interesting to note that ambient temperature influences the effects of maternal deprivation on the circadian clock. Ambient temperature in the neonatal period might reset the circadian clock, which is not the case in adulthood.

The mechanism by which maternal deprivation resets the circadian clock is unknown. Maternal deprivation changes the pup circadian rhythms in clock gene expression in the SCN (Ohta et al. 2003). Therefore, a nonphotic signal may reach the SCN and change the dynamics of autofeedback loop of clock genes, which is believed to be the mechanism of circadian rhythm generation (Reppert and Weaver 2002). However, neither the input pathway to the SCN nor the signal transduction mechanisms in the SCN cells associated with maternal deprivation is understood.

3. Effects of Maternal Deprivation on Basal and Stress-Induced Plasma Corticosterone Levels in Adulthood

Figure 2 illustrates the basal and stress-induced plasma corticosterone levels in MD12, MD3Lam, MD3Lpm, and the control at 8 weeks of age. Rats in individual cages were transferred to new individual cages of the same size, which contained fresh wood chips (novelty exposure). Two days before the novelty exposure, blood sampling was performed at 08:00 hours to determine the basal corticosterone level. Blood was sampled in freely moving rats from the tail tip.

The basal and stress-induced corticosterone levels were significantly elevated in MD12. The stress-induced but not the basal corticosterone levels increased in MD3Lam. Neither the stress-induced nor the basal levels were changed in MD3Lpm.

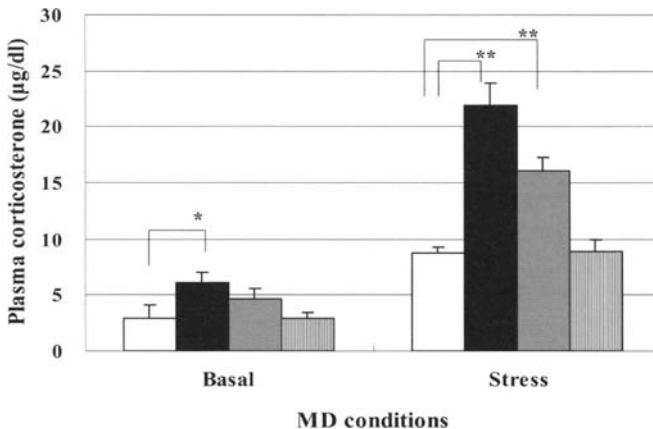


Fig. 2. The basal (*left*) and stress-induced (*right*) plasma corticosterone levels in adult rats subjected to MD12 (*filled columns*), MD3amL (*shaded columns*), MD3pmL (*striped columns*), and in the control (*open columns*). Values are expressed as the mean and SE ($n = 6$). Asterisks, statistically significant difference between the two groups linked with a bar; * $P < 0.05$, ** $P < 0.01$

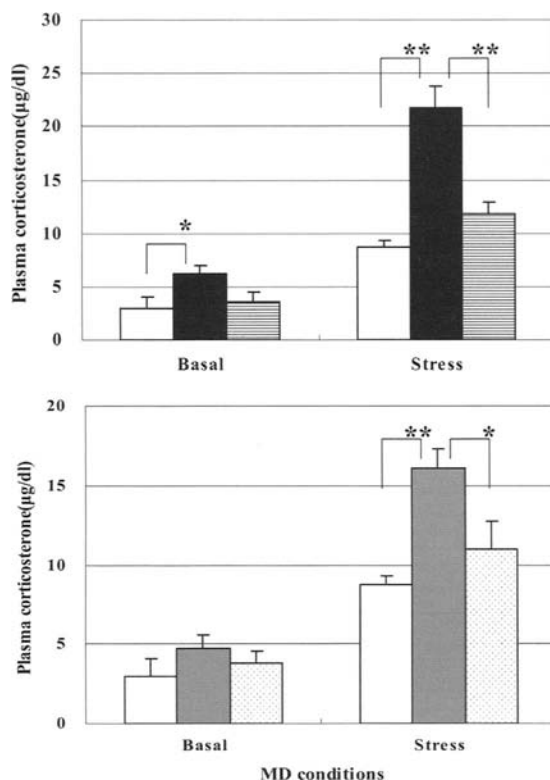


Fig. 3. The basal (*left*) and stress-induced (*right*) plasma corticosterone levels in adult rats subjected to MD12 (*filled columns*) and MD12T (*striped columns*) in the *upper panel*, and MD3amL (*shaded columns*) and MD3amLW (*dotted columns*) in the *lower panel*. The values of control rats are illustrated by *open columns*. Values are expressed as the mean and SE ($n = 6$). Asterisks, statistically significant difference between the two groups linked with a bar; * $P < 0.05$, ** $P < 0.01$

As reported previously, the stress responsiveness of grown-up pups was affected by maternal deprivation in the neonatal period, and the effects were dependent on the length and the time of day of maternal deprivation.

The influence of ambient temperature during maternal deprivation was also examined in the long-term effects of maternal deprivation on the HPA axis (Fig.3). Keeping the pups at 37°C during maternal deprivation canceled the effects of maternal deprivation in MD12 as well as in MD3am. The finding suggests that cooling pups during maternal deprivation is a critical factor to manifest the long-term effects of maternal deprivation in adulthood.

As for the mechanism of enhanced sensitivity of the HPA axis in grown-up pups, the negative feedback regulation of the HPA axis has been suggested to be modified

by maternal deprivation. Separation of neonatal pups from a nursing mother for 15 min decreased the paraventricular CRHmRNA and the stress-induced plasma corticosterone levels in adulthood (Liu et al. 1997). A brief separation led mothers to compensatory rich nursing, which may ultimately strengthen the negative feedback regulation of the HPA axis. On the other hand, a long-term separation such as in the present study may prevent enough nursing and exert an opposite effect.

4. Critical Periods for the Long-Term Effects of Maternal Deprivation on Circadian Clock and Stress Responsiveness of the HPA Axis

Nonphotic entrainment such as maternal entrainment seldom occurs, if at all, in adult rats. Actually, a critical period for maternal entrainment has been reported (Takahashi and Deguchi 1983). On the other hand, a critical period for the long-term effects of maternal deprivation on the HPA axis is not clear. Table 2 illustrates the activity ends and free-running periods of circadian locomotor rhythms at weaning in blind pups subjected to maternal deprivation of 12 h during different developmental stages: MD1w with maternal deprivation from P1 to P7, MD2w from P8 to P14, MD3w from P15 to P21 (Yamazaki et al. 2005b). The amount of phase shift of circadian locomotor rhythm was correlated with the stage of maternal deprivation. The largest phase shift up to 12 h was observed in MD1w, and the smallest phase shift of about 4 h in MD3w. The finding indicates that the effect of maternal deprivation on the pup circadian clock depends on the developmental stage, and suggests the existence of a critical period for maternal entrainment around weaning. On the other hand, the stress-induced elevation of plasma corticosterone level in adulthood was detected only in MD1w. The basal corticosterone level, but not the stress-induced corticosterone level, was significantly elevated in MD2w, and neither the basal hormone level nor the stress-induced hormone level was affected in MD3w (Fig 4). The finding indicates that maternal deprivation has a critical period for the long-term effect on the HPA axis, which is around postnatal day 7. The present findings suggest that the underlying mechanisms of the long-term effects of maternal deprivation on the circadian clock and on the PHA axis are different.

Table 2. Circadian phases and periods of locomotor rhythm at weaning in rats subjected to maternal deprivation during different developmental stages

MD conditions	Activity end (h)	Period (h)
MD1w	1.08 ± 0.52**	24.54 ± 0.03
MD2w	18.33 ± 0.54**	24.60 ± 0.03
MD3w	16.79 ± 0.33*	24.48 ± 0.05
Control	12.46 ± 0.31	24.46 ± 0.03

n = 6
* P < 0.05, ** P < 0.01 statistically significant difference from control

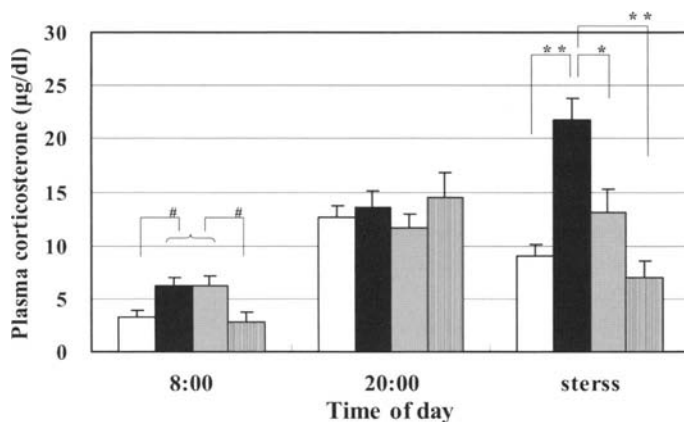


Fig. 4. Basal (8:00 and 20:00 hours) as well as stress-induced plasma corticosterone levels in adult rats subjected to maternal deprivation at different developmental stages; MD1w (*filled columns*), MD2w (*shaded columns*), MD3w (*striped columns*), and the control (*open columns*). Values are expressed as the mean and SE. Asterisks, statistically significant difference between the two groups linked with a bar; * $P < 0.05$, ** $P < 0.01$

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Part 2
Clinical Implications for PTSD and
Perspectives in Psychiatry

Current Perspectives on Clinical Studies of PTSD in Japan

Yoshiharu Kim

1. Introduction

This chapter provides a brief overview of recent Japanese clinical investigations on psychological trauma that have been conducted in affiliation with the National Institute of Mental Health (NIMH), the National Center of Neurology and Psychiatry and the Ministry of Health, Labor, and Welfare (MHLW). The studies focus on two main aspects of posttraumatic stress reaction: general human responses to traumatic events and the clinical nature of posttraumatic stress disorder (PTSD). The first aspect involves the diversity of human responses to the event and individual sociodemographic backgrounds, whereas the second deals with the commonality of traumatic reactions. The main focus of the discussion is on the diversity of human traumatic responses and there is also a brief overview of recent studies on the nature and treatment of PTSD.

2. The Great Hanshin-Awaji Earthquake

Japanese social and clinical concern for psychological trauma and PTSD arose after the Great Hanshin-Awaji Earthquake. The earthquake, with a magnitude of 7.6 on the Richter scale, occurred on 17 January 1995, at 5:46 am and caused the deaths of over 6400 people. If the earthquake had occurred during the day with more people on trains and with more cars on the roads, the number of the victims would have been far greater. The event followed the Unzen-Fugendake volcano eruption in 1990 and the Okushiri Earthquake in 1993. Not long after the Great Earthquake, an act of terrorism was perpetrated by an Omu religious cult with the use of poisonous sarin gas on the Tokyo subway system, which further increased social unrest.

The earthquake greatly afflicted Kobe, one of the largest and most modern cities of Japan. The sight of the ruined city was devastating to most Japanese people. De-

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stroying the belief that modern Japanese architecture would withstand powerful earthquakes, it evoked the common fear of earthquakes, rooted in the prior experience of the Great Kanto Earthquake of 1923, which, together with the subsequent fire, killed over 140 000 people in Tokyo and neighboring prefectures and caused damage amounting to 1.4 times the national budget at the time. People became aware that no one in the nation could be unaffected by a large earthquake. This awareness turned into reality in 2004 in the form of a great earthquake in Niigata, of which there was no prediction or warning. During an 18-day period, 4 earthquakes occurred with a magnitude greater than 6 on the Richter scale, and 13 occurred with a magnitude of 5 on the Richter scale, with the total number of tremors in that period being 721. The earthquake of Niigata is further examined later in this chapter.

In response to the augmented social need, mental healthcare was actively developed by the Psychiatry Department of Kobe University, the Hyogo Prefectural Center of Mental Health, local mental hospitals and clinics, and local governmental offices, which joined forces with a number of mental health teams from other areas of Japan. The mental health services provided at this time came to serve as the model for Japanese disaster mental healthcare, and the city of Kobe became a monumental center for PTSD research and education.

3. Peruvian Hostage Crisis in the Japanese Ambassador's Residence in 1996

Government-based PTSD research accelerated after the hostage crisis in Lima, Peru, which began on 17 December 1996 and lasted until its resolution on 22 April 1997. During the crisis, the Japanese government sent a medical team to treat the hostages upon their release, although the team initially lacked a psychiatrist. On the day the hostages were released, a team of psychiatrists, including the author, were sent to Lima as members of the governmental medical team to care for the liberated hostages and accompany the Japanese Ambassador on the flight back to Japan (Kim 1998).

In the lead-up to the crisis, several hundred people were invited to the birthday celebration of the Japanese emperor. The party guests were captured in the embassy garden, and held hostage in the house of the Japanese ambassador, where he lived with his wife and several staff members. The stressors experienced by the hostages varied according to the time course of the event. In the initial phase, the strongest stressor was the attack itself by the terrorists. Other stressors included lack of sleeping space and toilet facilities. Some hostages experienced psychological reactions such as feelings of confusion, anger, and disbelief. In one case, a sort of delusional idea was observed: a hostage had seen his old friend of 20 years that very morning and thought after the captivation that this had been an omen of the event.

In the later phase of the hostage crisis, due to the months-long duration of the event, both the hostages and terrorists were in a state termed *Provisiorium ohne*

Termin, or endless moratorium, and they grew tired and disorganized. Some hostages increasingly suffered from depression and fatigue, started to lose hope, and avoided thinking of the future. One hostage stated he decided to go into “hibernation,” although it is unclear whether this hibernation served as an intentional coping strategy or was itself a psychological reaction.

At last on 22 April 1997, the hostages were released amidst explosions and gunfire, contrary to the belief that they would be set free through peaceful negotiation. Most Japanese hostages witnessed the gunshots and escaped the subsequent fire on the ground floor by fleeing to the second floor, where they found the emergency door to be locked. Several minutes were spent in an attempt to open the locked door. While hearing gunshots and sensing the heat of the fire, some hostages were convinced that death was near. However, they finally opened the door and jumped from the roof, an act that left some of the escaped hostages with broken legs.

After the liberation, the author, together with Dr. Kasahara from the International Medical Center of Japan, who had been deployed to Peru during the time of the crisis, and Dr. Konishi from Tokyo Medical and Dental University, interviewed the freed Japanese hostages and followed-up on them after 1 year. The first interview revealed that insomnia, anxiety, and irritation were all common. Some terrorists were teenagers who could not go to school and lacked any knowledge of political issues, but were involved in the operation just for money. They showed a naive attachment to some Japanese hostages, even saying they would come to Japan to attend school when the crisis ended. This phenomenon, now termed the “Lima syndrome,” is an attachment opposite to the “Stockholm syndrome.” A transient emotional disturbance was experienced among some Japanese exhostages when the terrorists, regardless of their age, were killed just after the liberation.

4. Government Strategy for Research on Psychological Trauma

In response to the governmental policy to promote effective trauma care, the then director of the National Center of Neurology and Psychiatry of Japan, Prof. Masahiro Sugita, decided to develop a research team to study PTSD in 1998 and assigned the author to preside over it. During subsequent years, the author chaired a number of research teams studying psychological trauma, not only for the sake of medical research in a narrow sense, but also to improve social education on PTSD and related psychological problems, as well as to educate the public about appropriate and typical responses to tragic events. Within the framework of the government-funded research teams, a number of significant studies and social activities were carried out.

4.1. Measurement

Some standardized measurement tools, including the Japanese version of the Clini-

cian-Administered PTSD Scale (CAPS) and the Impact of Event Scale-Revised (IES-R) (Asukai et al. 2002), are useful in the measurement of PTSD. In the Wakayama arsenic acid poisoning case, the prevalence of PTSD among citizens after the event was found to be 21% at 3 months, 10% at 6 months, and 10% at 12 months. This illustrates that after an event in which a large percentage of the residents were killed, the prevalence of PTSD decreases naturally but then remains at nearly the same level. In general, we can expect a natural recovery from PTSD, but at the same time we should be concerned with the population whose traumatic pathology persists (Kim 2002a).

4.2. Overseas Counterterrorism

Apart from the Peruvian hostage crisis, Japanese citizens have been involved in numerous traumatic events overseas, such as other hostage crises that occurred in Kyrgyzstan, the Philippines, and in South America, as well as accidents and criminal attacks. Prominent among these are the terrorist attacks of 11 September 2001 (9/11) in New York, which evoked serious concern from Japanese society and government. After 9/11, the MHLW and the National Center of Neurology and Psychiatry launched a telephone counseling service for the people who returned to Japan. This facility was managed by Prof. Konishi and the author. Guidelines for mental healthcare administered by the authors' team after an act of terrorism were uploaded to the website of the MHLW and the NIMH.

4.3. Domestic Violence

In 2001, a law regarding violence toward one's partner was enacted for the first time in Japan as a result of augmented social concern and efforts by advocates on this issue. Prior to that legislation, a support and research program for domestic violence victims sheltered in the Tokyo Women's Center had already been implemented and had served to shelter and reeducate prostitutes. Traumatic symptoms measured by the IES-R and general mental health assessed by the General Health Questionnaire-30 significantly decreased after 2 or 3 weeks of residence in the shelter. Of note, intrusion symptoms decreased more than hyperarousal and numbing symptoms (Yanagita et al. 2004), presumably because the victims were no longer fearful for the first time in several years that their partner would intrude into their rooms, due to 24-h guarded surveillance, night watch staff, and a threefold locking system.

The second phase of the program aimed at following up the victims after their discharge from the shelter, as well as supporting the children who accompany most victims. We found that all children witnessed the victimization of their mother, and a number of them were abused as well. There may also be serious problems with attachment and fostering that require continuous psychological care and social support. Additionally, aggression and neglect are not infrequently observed. We intend to continue providing services at Tokyo Women's Medical College and will provide

group care for victimized children as well.

4.4. Nagasaki Atomic Bomb Study

On 9 August 1945, the atomic bombing of Nagasaki caused 74 000 deaths and an additional 75 000 injuries. People in the afflicted area were registered by the Japanese government as atomic bomb victims in order to receive medical and financial assistance from the government, but residents outside the immediately afflicted area were displeased about being excluded from the registration. Actually the initial registered area had quite an irregular shape, due to the fact that there are mountains on the eastern and western edge of Nagasaki city. This caused the wind to blow from north to south; hence little radioactive substance reached beyond the mountains into the surrounding areas. The residents did not believe this explanation, even after the government research committee concluded that there indeed was no significant pollution in the surrounding areas. In 1998, after repeated efforts and failures to allow the people in the surrounding areas to register as atomic bomb victims, Nagasaki city reported on the traumatic anxiety experienced by the residents in those areas and made a claim for their need of mental health support. In response to this report, the Ministry of Health, Labor, and Welfare set up a committee, which assigned a research team chaired by Prof. Kikkawa, an emeritus Director of NIMH, Japan, and included the author as its secretary. The aim of the research was to investigate the mental health of the residents on the outskirts of the affected area, in order to determine whether there was substantial worsening of their state of mental health due to the atomic bomb tragedy, regardless of whether true pollution and atomic debris had reached them.

The subjects recruited from the surrounding area who witnessed the atomic bomb explosion were compared with control subjects who had moved into the same area more than 5 years after the bombing. The two groups were matched by sex, age, and area of residence. The turmoil of the bombing and the war had almost disappeared within 5 years, so that the two groups differed in terms of their experience with the bombing but were similar in most life events at the community level for several decades. As a result, the two groups had no significant difference in demographic features or life events except the war experience, because some of the controls had returned to Nagasaki from war. The GHQ-28 score and the proportion of high-risk victims with scores of more than 6 (average risk), or more than 14 (high risk), were higher in the subjects than the controls. A multivariate analysis showed that it was only the experience of the exposure to the atomic bombing that significantly correlated with mental health, and not the current stigma, life events, etc. Among the subjects, traumatic fear measured by the IES-R significantly correlated with the variables associated with the atomic bombing; namely, the distress caused by the explosion, the awareness of the subsequent radioactivity, and the lifelong fear of radioactive pollution. Strikingly, most of the subjects had poor knowledge of the nature of radioactivity and confused it with lightning. They believed that they had

been one third of the distance from the ground zero inside the location where the radioactivity actually diminished (Kim 2002b). These results will be published in detail elsewhere. Based on this finding, the Japanese government admitted that the residents suffered psychologically from exposure to the atomic bomb explosion and the awareness of radioactivity caused by the event, and it responded by subsidizing Nagasaki city and prefecture with mental health support.

4.5. Prolonged Exposure

The author's division of the NIMH, Japan, has started a clinical study of prolonged exposure (PE) therapy for PTSD. We invited Prof. Edna Foa to Japan in 2003 and 2004 to hold 4-day and 2-day workshops, respectively. We conducted PE for several PTSD patients under the supervision of Foa's team and witnessed improvement of the subjects on the CAPS, Beck Depression Inventory scale, and other psychometric scale scores. Functional magnetic resonance imaging revealed that Broca's area came to be activated after the PE, presumably reflecting cognitive processing of the traumatic experience.

4.6. Clinical Trial of Selective Serotonin Reuptake Inhibitors

Currently, no drug has been authorized for PTSD under the national health insurance system, which is the only form of medical insurance in Japan. Physicians have to use drugs such as selective serotonin reuptake inhibitors (SSRIs), which fall under international guidelines, as the first choice for the treatment of PTSD. The author chairs a clinical trial of an SSRI, paroxetine, and has gathered supportive data.

4.7. Suppression of Cellular Immunity in Past PTSD

High rates of medical morbidity have been reported with PTSD. We investigated immune function in subjects with remitted PTSD (Kawamura et al. 2001). The initial study group was composed of 1550 Japanese male workers. The IES-R and the Diagnostic Interview Schedule for DSM-IV identified 12 workers with a past history of PTSD. These men were matched in age and smoking habits, which affect immunity, to 48 comparison subjects who had similar stressful life experiences but no current or past history of PTSD. The number of lymphocytes, number of T cells, NK cell activity, and total amounts of interferon- γ and interleukin-4 were significantly lower in the 12 men with a past history of PTSD. We concluded that PTSD leaves a long-lasting immunosuppression and has long-term implications for health.

4.8. *Guidelines and Academic Association*

After several years of struggling to promote effective care for traumatized people in Japan, substantial social, medical, and legislative recognition of psychological trauma has emerged. In 2000, the Japanese Society for Traumatic Stress Studies was founded. Its aim is not only to promote academic research but also to develop ties among clinicians who are responsible for the care of those who are victimized. In December 2004, the victims' rights law was enacted, which makes the government responsible for providing treatment to psychologically traumatized victims.

Our efforts to promote trauma care also resulted in effective disaster mental health activities in response to the Niigata great earthquake. Reflecting the accumulated experience and education since the Great Hanshin-Awaji Earthquake in 1995, more than 100 mental care teams gathered from various parts of Japan and worked under an organization directed by the Niigata prefecture government and Mental Health and Welfare Center of Niigata. There was no longer a fuss over PTSD or an obsessive urge for psychological debriefing, which was quite reasonable given that the response to traumatic events includes a wide range of mental disorders, including those that meet the classical notion of psychogenic reaction (Kim 2003). In other words, disaster mental healthcare was suitably incorporated into ordinary medical activity.

5. Conclusion

Our future aim is to develop an enduring, national-level system for trauma care and research, such as the National Center for PTSD in the USA. Trauma care and PTSD treatment should be emphasized not only when disaster strikes, but also in daily medical activity and community mental healthcare. PTSD should be "normalized" in the medical field and discussed alongside depression, panic disorders, and phobias. To achieve this objective, a greater array of evidence-based clinical studies and biological clarification of the human response to trauma will be needed.

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Psychosocial and Genetic Susceptibility to Posttraumatic Stress Disorder

Min-Soo Lee and Heon-Jeong Lee

1. Introduction

Posttraumatic stress disorder (PTSD) is an often-disabling psychiatric disorder that can result from exposure to trauma. PTSD develops in only a subset of persons exposed to traumatic stress, suggesting that individual differences influence the susceptibility to PTSD. In other words, individuals with prior vulnerability have a higher risk of developing PTSD upon exposure to a traumatic event. In this chapter, we introduce studies of our group on personality characteristics and genetic variants influencing PTSD susceptibility.

2. Psychosocial Factors

The psychiatric symptoms of disaster victims vary according to their social, cultural, and personal backgrounds. A recently completed report documented a definite dose-response relationship between the intensity of trauma and PTSD (McFarlane 1989). However, the fact that most of those exposed even to severe trauma do not show many stress disorder symptoms suggests the need to consider other possible factors. A study of Vietnam War veterans found that only 4% of uninjured veterans suffered from PTSD compared with 20% of the veterans who received war injuries (Breslau and Davis 1987). Another study found that although there was no association between exposure to combat and PTSD severity, a relationship did exist when combat led to the death of companions or involved torture (Yehuda et al. 1992). However, a contrasting report found that the severity of PTSD was less related to the experience of trauma itself, and more related to the individual's vulnerability or predisposition prior to the onset of stress (Perry et al. 1992). These results suggest that in addition to the severity of trauma, psychological vulnerability can affect the degree of PTSD.

Several studies of PTSD using the Minnesota multiphasic personality inventory

(MMPI) (Hathaway and Mckinley 1983) have collectively shown elevations on scales measuring both neurotic and psychotic symptoms, as well as on the F (infrequency) scale. Kinzie et al. (1984) reported significant Sc and D scale elevations in 13 Cambodian refugees, although Kinzie's subjects were housed in a refugee camp for 2–4 years and did not exhibit any acute symptoms. However, Stucker et al. (1991) reported D, Hs, Sc, and Pt scale elevations of more than 70 points in a comparative study of 22 Korean War veterans who had been imprisoned and a veteran control group, leading to the conclusion that MMPI and the severity of trauma are correlated. Among the validity scales, the F scale showed the greatest increase.

2.1. Sampoong Disaster Victim Study

The Sampoong department store in Seoul collapsed on 29 June 1995, leaving 458 people dead and 941 injured. After 5 months, 1231 survivors of the Sampoong department store collapse visited the Korea University Medical Center for general medical evaluations; out of these, 681 visited the Department of Psychiatry for psychiatric evaluation. After excluding those with missing data, we (Lee et al. 2004) analyzed the clinical data of 617 patients (472 females, 145 males) who underwent psychiatric examination. Each subject was evaluated using the psychiatric evaluation form and the MMPI. Two psychiatrists grouped the patients into four symptom severity categories: Normal, Mild, Moderate, and Severe. The Normal category comprised subjects with transient symptoms or no symptoms at all. The Mild category comprised subjects who experienced minor but continuing difficulties that affected their everyday life. Subjects who were unable to work were classified as Moderate, and subjects who were hospitalized, under the care of others, or who showed psychotic symptoms were classified as Severe. Correlations between symptom severity and potential factors of influence were examined. Sociological factors such as sex, age, marital status, and education were first considered; whether subjects were rendered unconscious was also a prime consideration. A third set of factors included those thought to affect the degree of mental trauma, including lapse of time prior to rescue, death of family member(s), and direct exposure to dead bodies.

2.1.1. Psychosocial Factors Affecting Symptoms

Sex

The majority of male and female subjects suffered from mildly severe symptoms (44.1% of males, 47.7% of females) and moderately severe symptoms (37.9% of males, 39.6% of females). There was no significant statistical difference in symptom severity between men and women.

Age

The mean ages of the Normal (58 subjects), Mild (289 subjects), Moderate (242 subjects), and Severe (28 subjects) groups were 27.9, 29.6, 34.7, and 31.3 years re-

Table 1. Severity of psychiatric symptoms and marital status

Marital status	Severity of psychiatric symptoms				Total
	Normal	Mild	Moderate	Severe	
Unmarried	37 (10.6%)	178 (51.0%)	116 (33.2%)	18 (5.2%)	349 (100%)
Married	20 (8.4%)	102 (43.0%)	105 (44.3%)	10 (4.2%)	237 (100%)
Divorced	0 (0%)	4 (28.6%)	10 (71.4%)	0 (0%)	14 (100%)
Widowed	1 (5.9%)	5 (29.4%)	11 (64.7%)	0 (0%)	17 (100%)
Total	58 (9.4%)	289 (46.8%)	242 (39.2%)	28 (4.5%)	617 (100%)

$\chi^2 = 21.4$, $df = 9$, $P = 0.011$

Table 2. Severity of psychiatric symptoms and educational level

Education	Severity of psychiatric symptoms				Total
	Normal	Mild	Moderate	Severe	
No education	1 (1.7%)	8 (80%)	1 (10.0%)	0 (0%)	10 (100%)
Elementary school	2 (5.9%)	8 (23.5%)	24 (70.6%)	0 (0%)	34 (100%)
Middle school	2 (4.4%)	11 (24.4%)	29 (64.4%)	3 (6.7%)	45 (100%)
High school	38 (10.1%)	191 (50.7%)	131 (34.7%)	17 (4.5%)	377 (100%)
Further education	15 (9.9%)	71 (47.0%)	57 (37.7%)	8 (5.3%)	151 (100%)
Total	58 (9.4%)	289 (46.8%)	242 (39.2%)	28 (4.5%)	617 (100%)

Likelihood ratio $\chi^2 = 38.7$, $df = 12$, $P < 0.001$

spectively. Symptoms became more severe as age increased, but the differences were not significant.

Marital Status

There was a significant statistical difference in the severity of symptoms among the four marital status groups ($P = 0.011$, Table 1). The Single group had a larger ratio of normal or mild symptom patients versus moderate or severe symptom patients than did the married group, whereas the divorced and widowed groups contained more severe symptom patients.

Education Level

There was a significant statistical difference in the severity of symptoms among the five educational status groups ($P < 0.001$, Table 2). High school and college graduates had similar distributions of severity. However, middle school graduates and elementary school graduates showed higher overall symptom severity than high school and college graduates.

Table 3. Severity of psychiatric symptoms and Level of loss of consciousness

Loss of consciousness	Severity of psychiatric symptoms				Total
	Normal	Mild	Moderate	Severe	
No answer ^a	2 (6.7%)	13 (43.3%)	12 (40.0%)	3 (10.0%)	30 (100%)
None	43 (12.5%)	179 (51.9%)	113 (32.8%)	10 (2.9%)	345 (100%)
Less than 5 min	5 (5.8%)	38 (44.2%)	40 (46.5%)	3 (3.5%)	86 (100%)
5–30 min	2 (3.0%)	29 (43.9%)	32 (48.5%)	3 (4.5%)	66 (100%)
30–60 min	2 (5.1%)	14 (35.9%)	22 (56.4%)	1 (2.6%)	39 (100%)
1–6 h	4 (10.5%)	13 (34.2%)	16 (42.1%)	5 (13.2%)	38 (100%)
6–12 h	0 (0%)	1 (33.3%)	2 (66.7%)	0 (0%)	3 (100%)
12–24 h	0 (0%)	0 (0%)	1 (50.0%)	1 (50.0%)	2 (100%)
Greater than 24 h	0 (0%)	2 (25.0%)	4 (50.0%)	2 (25.0%)	8 (100%)
Total	58 (9.4%)	289 (46.8%)	242 (39.2%)	28 (4.5%)	617 (100%)

^aNo answer group was not included in the analysis
linear-by-linear association $\chi^2 = 26.8$, $df = 1$, $P < 0.001$

Table 4. Severity of psychiatric symptoms and duration until rescue

Duration until rescue	Severity of psychiatric symptoms				Total
	Normal	Mild	Moderate	Severe	
Less than 5 min	22 (15.7%)	67 (47.9%)	43 (30.7%)	8 (5.7%)	140 (100%)
5–10 min	13 (30.2%)	17 (39.5%)	11 (25.6%)	2 (4.7%)	43 (100%)
10–30 min	9 (8.1%)	64 (57.7%)	33 (29.7%)	5 (4.5%)	111 (100%)
30–60 min	6 (3.9%)	80 (51.6%)	64 (41.3%)	5 (3.2%)	155 (100%)
1–5 h	7 (6.3%)	52 (46.4%)	48 (42.9%)	5 (4.5%)	112 (100%)
5–12 h	1 (3.8%)	8 (30.8%)	15 (57.7%)	2 (7.7%)	26 (100%)
12–24 h	0 (0%)	0 (0%)	4 (80.0%)	1 (20.0%)	5 (100%)
1–7 days	0 (0%)	1 (4.3%)	22 (95.7%)	0 (0%)	23 (100%)
1–2 weeks	0 (0%)	0 (0%)	2 (100%)	0 (0%)	2 (100%)
Total	58 (9.4%)	289 (46.8%)	242 (39.2%)	28 (4.5%)	617 (100%)

Linear-by-linear association $\chi^2 = 32.3$, $df = 1$, $P < 0.001$

2.1.2 Trauma Characteristics Affecting Symptoms

Loss of Consciousness

There was a significant statistical difference in the severity of symptoms among the seven groups ranked according to duration of loss of consciousness ($P < 0.001$, Table 3). Thirty subjects did not answer the question about loss of consciousness. The others were divided into the groups of Conscious or Unconscious based on whether or not they had lost consciousness during the collapse. The Conscious group comprised 345 members of the study group. The Unconscious group contained 242 subjects. Loss of consciousness was associated with more severe psychiatric symptoms.

Duration Until Rescue

There was a significant statistical difference in the severity of symptoms among the nine groups ranked according to time spent trapped or buried at the disaster site ($P < 0.001$, Table 4). Symptom severity increased as rescue time lengthened.

Witnessing Death

Subjects who were directly exposed to dead bodies were not significantly affected by the experience. However, when subjects were directly exposed to the bodies of relatives or close friends, symptoms were more severe than in cases of exposure to

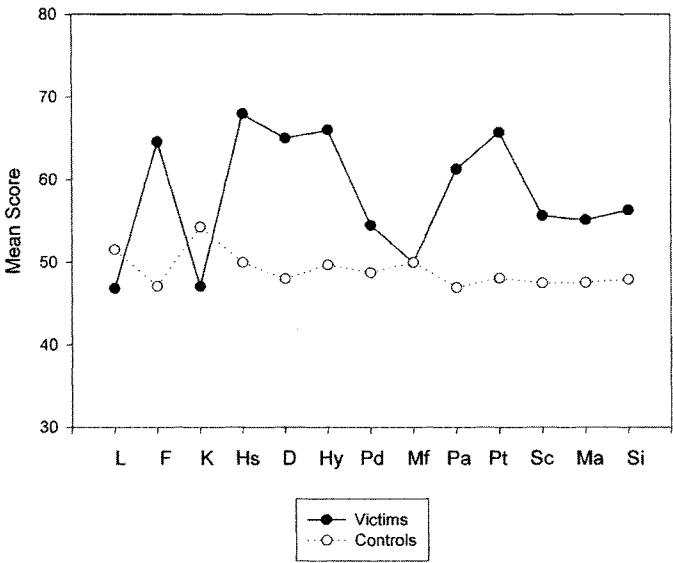


Fig. 1. Comparison between the Minnesota multiphasic personality inventory (MMPI) profiles of Sampoong disaster survivors and normal controls

the bodies of strangers or even colleagues. Subjects who directly witnessed the act of death at the disaster site also had slightly more severe symptoms.

2.1.3. Psychological Factors

In addition to the physical injuries and financial loss associated with natural and man-made disasters, witnesses and participants involved in disaster incidents such as war, air traffic accidents, earthquakes, or tornadoes frequently experience psychological trauma. Sustaining injuries from a building collapse is also a disaster experience that can lead to psychological consequences. When the mean MMPI scale scores of Sampoong disaster victims were compared with a normative control group, all scales except *Mf* were significantly different (Fig. 1). The MMPI test results in the victims showed the F-1-2-3-6-7 profile pattern. These results are comparable with the profile pattern of 1-2-3-7-8 reported by Keh and Choi (1993) in a MMPI study of 50 patients who were diagnosed as PTSD following their involvement in traffic accidents. The Sampoong MMPI profile featured an elevated *Pa* scale in contrast with the elevated *Sc* scale of a traffic accident group, implying that a building collapse might affect symptoms differently than traffic accidents and other traumatic experiences. In other words, the survivors from the collapse might have become less trusting because they experienced the collapse of a building that was regarded as a safe place for rest and shopping. This hypothesis is supported by hypersensitivity shown by most subjects during interviews.

Our results for the Sampoong disaster victims showing elevated *F*, neurotic, and psychotic scales are consistent with the hypothesis that PTSD is not a simple anxiety disorder but a complex disorder of emotion and cognition as suggested by McCormack et al. (1990). A formula was successfully developed to differentiate survivors from controls; *L*, *F*, *K*, *Hs*, *D*, *Hy*, *Pt*, *Sc*, and *Ma* scales were found to be valid for the discrimination in our study. A discriminant analysis with 45 PTSD patients by Eun et al. (1994) showed that *L*, *F*, *K*, *Hs*, *D*, *Mf*, *Pt*, and *Si* scales were valid for the discrimination, and that higher *D*, *Mf*, and *Pt* scales and lower *K* and *Si* scales were better in the discrimination. Our study showed that the higher *F*, *Pt*, and *Hy* scales and the lower *Sc*, *L*, and *K* scales had better discriminating potential. However, both studies showed comparable overall discrimination probabilities of 92% and 89.84% respectively, and they thereby confirmed the validity of MMPI for differentiating disaster victims from controls.

Cluster analysis based upon MMPI T scores in the Sampoong disaster subjects yielded three discriminated groups. The first group was a normal reaction group, which did not show any particular symptoms. The second group was a neurotic group, which had high levels of anxiety and depression and a tendency toward somatization. The third group was a symptom-complaint group, which showed a uniquely elevated *F* score and entirely elevated scores in the clinical scales. Analyses using 16 personality factors revealed a normal group that was psychologically healthy, and a symptomatic group that was quite vulnerable and had substantial psychopathology (Kim et al. 1997).

Psychiatric symptoms precipitated by disaster experiences vary with social, cul-

tural, and personal backgrounds. Kim and Ahn (1996) surveyed and interviewed 102 disaster victims and 42 family members of victims and reported that most victims exhibited neuropsychiatric symptoms, but they did not list the symptoms. A study conducted with 124 survivors of Nazi prison camps found that sleep disturbance, recurring nightmares, and distress from retrospection were frequent symptoms, whereas psychogenic amnesia and fear of recurrence were not. Vietnam War veterans experienced flashbacks more frequently than the survivors of other disasters (Kuch and Cox 1992). Korean War veterans held captive during the war experienced more serious difficulty with concentration, mistrust of others, lethargy, and expressing emotion than did veterans who avoided capture (Stucker et al. 1991). Cambodian refugee camp veterans exhibited symptoms of avoidance, blunting, hyperactive startle, and nightmares (Kinzie et al. 1984). Zeitlin et al. (1993) found that rape victims showed frequent alexithymia and that repeated rape led to more pronounced symptoms.

3. Genetic Susceptibility

Twin studies of Vietnam veterans have shown a significant genetic contribution to PTSD (Koenen et al. 2003). True et al. (1993) studied 4042 twin pairs from the Vietnam Era Twin Registry and found that inherited factors accounted for up to 32% of the variance of PTSD symptoms, above and beyond the contribution of trauma intensity. Xian et al. (2000) examined 3304 twin pairs of the Vietnam Twin Registry and similarly found 35.3% inherited liability to develop PTSD upon combat exposure, 20% of which pertained specifically to PTSD, 15.3% of which was common to both PTSD and substance dependence.

The fact that the underlying genotypic vulnerability to PTSD is only expressed following trauma exposure limits the usefulness of family-based linkage approaches. In contrast to other major psychiatric disorders, large studies for the search of underlying genes have not been described in PTSD to date. Complementary approaches for locating involved genes include association-based studies employing case-control or parental genotypes for transmission disequilibrium analysis and quantitative trait loci studies in animal models.

The genetic basis of vulnerability to PTSD is hardly known at this point. Until now a positive association has been reported for only two genetic polymorphisms, Taq-I RFLP of the D2 dopamine receptor (DRD2) gene (Comings et al. 1996), and the dopamine transporter gene (DAT1) (Segman et al. 2002). There is even substantial controversy over these findings. Beyond their conflicting results, these studies are limited by their small sample sizes, by the salient presence of comorbid substance abuse, and by the fact that the occurrence of a traumatic event was not evaluated among control subjects in the second and larger study. Some control subjects may have undergone a traumatic event whereas others have not been exposed to a trauma.

3.1. Two Multicenter Collaboration Studies

We have performed two multicenter collaboration studies since 2000, involving different PTSD patients. PTSD patients were recruited from existing clinical populations visiting collaborating centers. Both studies included approximately 100 PTSD patients and normal controls. Trained psychiatrists examined all subjects using the Structured Clinical Interview for DSM-IV–Korean version (SCID-K) (Han and Hong 2000). We examined the possible association between several candidate gene polymorphisms and PTSD in different groups. The selected candidate genes were the serotonin-transporter-linked-polymorphic region (5-HTTLPR), the dopamine D2 receptor (DRD2), the serotonin 2A receptor (5-HTR2A) gene –1438A/G polymorphism, and the brain-derived neurotrophic factor (BDNF) gene Val66Met polymorphism.

3.1.1. Serotonin Transporter Gene (5-HTTLPR)

Serotonin is regarded as important in complex neuronal communication (Lesch and Mossner 1998). Although the role of the serotonergic transmission in the pathophysiology of PTSD is still unclear, the involvement of serotonin in the expression of PTSD symptoms is suggested by its role in regulating mood, arousal, or sleep (Jacobs 1991) as well as by the clinical similarity of PTSD and other psychiatric disorders/behaviors that have been linked to serotonin dysfunction (Davis et al. 1999; Brady et al. 2000). In addition, there is evidence for the involvement of serotonin in the pathogenesis of PTSD from clinical studies demonstrating beneficial effects of drugs that enhance serotonergic neurotransmission, in particular, tricyclic antidepressants, monoamine oxidase inhibitors, and selective serotonin reuptake inhibitors (Fichtner et al. 1994; Davis et al. 1999).

The serotonin transporter regulates the magnitude and duration of serotonergic neurotransmission and serves as an initial target site for antidepressants (Graham and Langer 1992; Schloss and Williams 1998). A serotonin transporter-linked polymorphic region (5-HTTLPR) within the promoter of the serotonin transporter gene has been identified. In vitro basal serotonin transporter activity in carriers of the 5-HTTLPR long (l) allele was found to be more than twice that in carriers of the short (s) allele, suggesting that serotonin transporter gene transcription is modulated by such variants (Heils et al. 1996; Lesch et al. 1996).

In our study, the genotypes of 5-HTTLPR were classified into two groups: carriers of the l allele (l/l and l/s genotypes) and carriers of the s/s genotype. There were marginal differences in genotypes between the two groups, and the allele frequency was significantly different ($P < 0.05$). When the genotypes of 5-HTTLPR were dichotomized into l/l+l/s and s/s groups, the frequency of the s/s genotype was significantly higher in patients with PTSD ($P < 0.05$) (Lee et al. 2005).

3.1.2. Serotonin 2A Receptor Gene (5-HTR2A)

The serotonin 2A receptor (5-HTR2A) has received much attention in psychiatric research, because several postmortem studies have suggested 5-HTR2A upregulation in the frontal cortex of depressed patients and suicide victims (Yates et al. 1990; Arora and Meltzer 1989). In addition, it was recently reported that the -1438 A/G polymorphism in the promoter region of the 5-HTR2A gene is significantly associated with bipolar disorder (Chee et al. 2001), seasonal affective disorder (Enoch et al. 1999), anorexia nervosa (Enoch et al. 1998), and major depressive disorder (Choi et al. 2004). We examined the possible association between the 5-HTR2A gene -1438 A/G polymorphism and vulnerability to PTSD in the Korean population. No significant differences were found between the genotypes and allele frequencies of PTSD patients and normal controls. However, a marginal difference was found between the two groups in terms of allele carrier frequencies ($P = 0.093$). On comparing men in the two groups, no significant difference was found with respect to genotypes, or allele and allele carrier frequencies ($P > 0.05$). However, a similar comparison of females, showed that PTSD patients had a significantly higher frequency of the noncarrier of the A allele (the GG genotype) than normal controls ($P = 0.036$).

3.1.3. Brain-Derived Neurotrophic Factor Gene

There is some evidence that brain-derived neurotrophic factor (BDNF) is associated with stress-related psychiatric conditions. Recently, Rasmusson et al. (2002) examined the effects of footshock stress and reexposure to cues previously associated with footshock on expression of BDNF mRNA in the hippocampus of male rats. They reported that psychological, as well as unconditioned physical stress can decrease hippocampal BDNF mRNA.

BDNF is a member of the neurotrophin family of peptides. As other neurotrophins, BDNF plays an important role in neuronal growth and differentiation during development, and it also contributes to the survival, function, and plasticity of neurons in the adult brain (Lewin and Barde 1996). BDNF is also thought to play a role in the molecular and behavioral responses to stress (Duman et al. 1997; Duman et al. 2000). Moreover, downregulation of BDNF could contribute to the hippocampal volume reduction observed in psychiatric illness such as PTSD and depression (Bremner et al. 1995; Mervaala et al. 2000; Vakili et al. 2000). From these findings, the BDNF gene is regarded as a candidate gene for susceptibility of PTSD.

We classified the genotypes of BDNF Val66Met polymorphism into two groups: carriers of the Met66 allele (Met/Met and Met/Val genotypes) and carriers of the Val/Val genotype, because the Met66 allele has been reported to have a lower activity-dependent secretion of BDNF and poorer human memory and hippocampal function (Egan et al. 2003; Rybakowski et al. 2003). The genotype and allele frequencies for the BDNF gene polymorphism did not differ between the two groups ($P > 0.05$).

3.1.4. Dopamine 2 Receptor Gene (DRD2)

Stress affects central dopamine systems, and dopaminergic innervation of the pre-frontal cortex is highly sensitive to stress (Thierry et al. 1976; Deutch and Roth 1990). It has been hypothesized that patients with PTSD suffer from a functional deficit in dopaminergic tone that compromises their ability to develop effective coping strategies for dealing with trauma (Deutch and Young 1995). Comings et al. (1991) first reported a significant association of the DRD2 A1 allele with PTSD. Comings et al. (1996) also reported a genetic association between the DRD2 A1 allele and PTSD in a sample of 37 subjects. This study was framed as a comparison of combat-exposed subjects with or without PTSD on an addiction treatment unit. We compared the allele and genotype frequencies between the PTSD group and normal controls. The genotype and allele frequencies for the DRD2 gene A1 polymorphism did not differ between the two groups ($P > 0.05$).

4. Psychosocial Versus Genetic Susceptibility

The etiology of PTSD is complex and multifactorial. Importantly, exposure to a traumatic event does not fully explain the occurrence of the disorder. Exposure, in fact, triggers a cascade of biological events that ultimately lead to the occurrence of chronic PTSD. Individuals with prior vulnerability are at higher risk for developing PTSD upon exposure to a traumatic event. As we have presented, personal characteristics such as lower educational level and divorced or widowed marital status, and F and clinical scale elevations on the MMPI, were associated with the psychiatric symptoms of disaster survivors. Psychological vulnerability can affect the severity of PTSD. Alternatively, however, traumatic experiences may be causes of psychological changes. Hence, prospective and longitudinal approaches are needed to solve this issue.

In previous studies, the genotype and allele frequencies of candidate genes were analyzed in PTSD patients and unrelated healthy controls using a case-control design. We found that 5-HTTLPR *s/s* genotype and the GG genotype of 5-HTR2A gene were significantly higher in PTSD patients than in normal controls. However, the levels of statistical significance were not high. The genotype and allele frequencies for the BDNF and DRD2 gene polymorphism did not differ when comparing PTSD patients with controls. These findings suggest that the BDNF and DRD2 polymorphism do not play a major role in the susceptibility of PTSD in the Korean population.

In the future, it will also be necessary to evaluate the possible involvement of as-yet-uncovered gene(s) that influence susceptibility to PTSD, and to consider the possibility of gene-gene, gene-personality, and gene-environment interactions.

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Intrusion in Women with Breast Cancer

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1. Introduction

Intrusion, a core symptom of posttraumatic stress disorder (PTSD), has begun to receive research as well as clinical attention in individuals with cancer. “When the mastectomy scar on my breast smarts, I remember the distressing scene when I was informed that I had cancer, and the image of death popped into my head.” This is the complaint of a woman who has survived for 5 years after the cancer diagnosis. Such thoughts are typical of breast cancer survivors; this patient describes this cancer experience as “engraved” in her brain.

The psychiatric impact of cancer has been a subject of active research interest and is being increasingly recognized in clinical practice. One of the primary reasons for this growing interest is the increasing number of cancer survivors. The fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) sets forth that a diagnosis of PTSD can be made under circumstances when a patient is traumatized by the experience of a life-threatening illness. Despite this addition, the applicability of this framework to PTSD secondary to cancer is potentially problematic because of the ambiguity of the stressors. More specifically, it is difficult to disentangle the effects of being told about the diagnosis from the ongoing, often aversive side effects of medical treatment, as well as future concerns about recurrence and mortality (Kangas et al. 2002). Whereas a formal diagnosis of PTSD appears to be limited to a small minority (3%–6%) of adult early-stage breast cancer survivors, intrusion is a more common feature (Alter et al. 1996; Andrykowski et al. 1998; Green et al. 1998; Matsuoka et al. 2005). It has been suggested that intrusion is associated with the presence of persistent depression or anxiety (Epping-Jordan et al. 1999; Nordin and Glimelius 1999), poor psychological adjustment (Matsuoka et al. 2002), and poor quality of life (Cordova et al. 1995). Furthermore, it has been reported that even in the general population, patients with subthreshold PTSD, in whom the most common symptom is intrusion, suffered from comorbid depression, suicidal ideation, and impairment of daily life activities (Marshall et al. 2001). Thus, early evaluation and intervention for such symptoms among cancer patients

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may be an important component of public health efforts targeted at mitigating the effects of disclosure of cancer diagnosis and cancer treatment. This chapter focuses on conceptual issues pertaining to cancer-related intrusion, critiques the empirical literature on PTSD associated with cancer, and presents plausible neurobiological mechanisms, useful neuroimaging studies, and the future directions of cancer-related intrusion.

2. Empirical Review of Cancer-Related Intrusion

2.1. Epidemiology

The majority of prevalence studies to date have focused on women diagnosed predominantly with early-stage to moderately advanced-stage breast cancer and have employed cross-sectional methodologies. Among studies that have utilized the Structured Clinical Interview for DSM-IV, the prevalence of reexperiencing the trauma, such as intrusive recollections, have ranged from 11% to 45% (Alter et al. 1996; Andrykowski et al. 1998; Green et al. 1998; Matsuoka et al. 2005). Green et al. (1998) used strict diagnostic decision rules that avoided counting future-oriented concerns as reexperiencing symptoms (which might result in false-positive diagnosis of PTSD diagnoses). Using these criteria, they found that only 1.9% of 160 breast cancer survivors could be diagnosed as cases of PTSD; however, 20% of the cases had current intrusion. Previous studies suggest that intrusion occurs at a relatively high prevalence.

2.2. Course

In terms of longitudinal research, Tjemsland et al. (1998) estimated that 18% of breast cancer patients reported a high level of intrusion on the basis of the Impact of Event Scale, 6 weeks postoperatively, and 9% reported intrusion 12 months postoperatively. In a cross-sectional study, Kornblith et al. (2003) revealed that 12% of women who survived for 18 years reported intrusion on the basis of the PTSD Checklist, Civilian version.

2.3. Predictive and Associated Factors

Across studies in cancer-related PTSD, sociodemographic variables (younger age, lower income, perceived low social support) (Cordova et al. 1995; Green et al. 2000), biomedical factors (premorbid health problems, later-stage diagnosis) (Andrykowski and Cordova 1998; Green et al. 2000; Tjemsland et al. 1998), and psychological factors (neuroticism, negative life events, psychiatric diagnosis, precancer trauma, trait anxiety) (Andrykowski and Cordova 1998; Bleiker et al. 2000;

Green et al. 2000; Tjemsland et al. 1998) have been reported to act as significant predictors of the development and severity of PTSD or PTSD symptoms in early-stage cancer survivors. In women with metastatic breast cancer, a shorter time since recurrence is predictive of intrusion and avoidance symptoms, and the size of the emotional support network is predictive of avoidance symptoms (Butler et al. 1999). However, the relative contribution of each of these factors to subthreshold PTSD development in early-stage cancer survivors has received little empirical attention (Gurevich et al. 2002). Recently, a study to identify the determinants of cancer-related intrusive recollections was reported (Matsuoka et al. 2005); in this study, a consecutive series of breast cancer patients at 3–15 months after surgery, consisting of subjects with current intrusive recollections and without intrusive recollections, were examined. Logistic regression analysis revealed that neuroticism [odds ratio (OR) = 1.27], precancer intrusive recollections about noncancer trauma (OR = 4.12), and the number of cancer patients among relatives by marriage (OR = 1.64) were final significant determinants, and that undergoing radiotherapy (OR = 0.46) was a protective factor. Bio-psycho-social factors contribute to intrusion, and have clinical implications that they can be used during screening and for early intervention.

3. Distinctive Features of Cancer-Related Intrusion

3.1. *Cancer as a Trauma*

The diagnosis and treatment of cancer is an extremely stressful experience for most individuals (Cordova and Andrykowski 2003). For example, the initial diagnosis about having a potentially deadly illness can be sudden and unexpected, and surgery can be invasive, painful, and disfiguring (Buckley et al. 2004). On the other hand, a minority (41%) among patients with breast cancer in the waiting room reported responding to cancer with intense fear, helplessness, or horror (DSM-IV-TR, A2 criterion) (Palmer et al. 2004). Although in many respects, the trauma of cancer resembles that of other traditional traumatic stressors (e.g., rape, combat, childhood sexual abuse), some distinguishing features are described below:

Internal threat. In contrast to external events, such as sexual and physical assault, combat, and natural disasters, cancer originates internally, within the body (Green et al. 1998; Gurevich et al. 2002; Kangas et al. 2002). Therefore, the subject cannot physically avoid the “stressor.” Furthermore, bodily signs or symptoms, such as postsurgical scars, pain, and unpleasant treatment also often serve as persistent reminders of the disease.

Future-oriented threat. The stressor causing cancer-related PTSD may also be distinctive, because the threat to one’s physical integrity is not necessarily immediate and the outcome of the stressor is future oriented.

Chronic threat. Although a key feature of PTSD is the persistence of the stress response even after disappearance of the stressor, in the case of cancer, it may be

difficult to distinguish symptom repetition from a new or current threat (Gurevich et al. 2002). Triggers for stress responses may include disclosure of the cancer diagnosis, treatment procedures, witnessing the adverse course of other patients, or even routine follow-up examinations (Gurevich et al. 2002). Recurrence of cancer may be even more traumatic than the initial disclosure of the diagnosis (Cella et al. 1990). Then this entire cycle of stress responses may be repeated.

Uncertain, intangible, and anticipatory threat. Cancer represents, in part, an “informational threat.” The intangible nature of this threat, as well as the uncertainty regarding the outcome of the disease may amplify the stress associated with the illness experience. The trajectory of cancer is often unpredictable, with long periods of remission interrupted by acute episodes that require palliation, and in addition, the impending encounter with death lingers throughout the course of the disease (Gurevich et al. 2002).

3.2. *Critical Issues in Cancer-Related Intrusion*

The DSM-IV description of reexperiencing symptoms implies that the intrusion and associated affect pertain to an event that has already occurred. This is potentially problematic in the context of cancer, because many intrusions are future-oriented fears about one’s health. There is evidence of this from one sample of breast cancer patients who reported that the information they received about their ongoing prognostic status was even more stressful than the intrusion associated with the treatment they had received (Green et al. 1998). This finding suggests that these women may be more prone to intrusions about anticipatory fears regarding their health status than to ruminating on past cancer experiences (Kangas et al. 2002).

Furthermore, Palmer et al. (2004) proposed another interpretation, that intrusion does not indicate any significant pathology among breast cancer survivors, as it represents normative cognitive processing of a stressor, and this cognitive processing of an ongoing health condition that holds the realistic threat of progression, side effects of treatment, or recurrence may be qualitatively different from the unbidden thoughts about the past experience. Deimling et al. (2002) also suggested that cancer-related intrusion may be interpreted as appropriate vigilance and effort to manage physical symptoms rather than as a maladaptive response.

Further study may be needed to examine the diagnostic usefulness of past-oriented versus future-oriented intrusions and to incorporate assessment of the content and temporal focus of cancer-related intrusions in order to differentiate normal processing from PTSD.

4. Neurobiological Mechanism Involved in Cancer-Related Intrusion

Emotional arousing or stressful situations produce several bodily changes, resulting from activation of the (nor)adrenergic system and hypothalamus-pituitary-adrenal (HPA) axis. Considerable evidence suggests that these systems act on consolidation of long-term memory for emotional experiences as well as constitute the neurobiological basis of memory disturbances in PTSD (Elzinga and Bremner 2002). These stress mechanisms are also appropriate to understand the neurobiological systems activated in cancer-related intrusion; therefore, this section focuses on these systems at first and then on recent neuroimaging studies in cancer-related intrusion.

4.1. The Noradrenergic System and the Amygdala

From the viewpoint of clinical evidence, retrieval of a traumatic event is accompanied by adrenaline release and this may further aggravate the traumatic memory (Elzinga and Bremner 2002). Several studies have suggested the role of noradrenaline in intrusive memories (Lemieux and Coe 1995; Yehuda et al. 1992). Evidence from both animal and human studies strongly suggests that (nor)adrenaline acts on the amygdalar β -adrenergic receptors to enhance memory (Cahill and McGaugh 1998; McGaugh 2000). In several laboratory studies, whereas increased noradrenergic activity has been shown to be associated with enhanced emotional memory (O'Carroll et al. 1999; Southwick et al. 2002), blockade of noradrenergic activity by propranolol infusion reduces the recall of an emotionally arousing story (Cahill et al. 1994; van Stegeren et al. 1998). The following experimental protocol developed by Cahill et al. (1994) has been widely used in human studies. Subjects are shown a series of slides with an emotionally arousing or neutral accompanying narrative, and 1 week later, a "surprise" memory test was conducted to assess the difference in memory storage between the emotional and neutral stories. The role of the amygdala in emotional memory has been highlighted by several positron emission tomography studies (Cahill et al. 1996; Hamann et al. 1999). For example, in the study conducted by Cahill et al. (1996), the glucose metabolic rate of the right amygdaloidal complex during the viewing of emotional films was highly correlated with the number of emotional films recalled, whereas no significant correlation was observed with the number of neutral films recalled. To summarize, it is suggested that the amygdala mediates the effects of emotion on memory.

4.2. The HPA Axis and the Hippocampus

As with noradrenaline, glucocorticoids (cortisol in primates), which are produced by the stress-responsive HPA axis, regulate hippocampal metabolism to enhance

memory (McGaugh 2000). A great deal of animal research has documented that mild glucocorticoid elevations enhance memory, whereas extreme elevations or deficiencies disrupt memory (McEwen and Sapolsky 1995). Human studies have shown either facilitation or impairment of explicit memory associated with elevation of cortisol release. Two studies showed facilitation of emotional memory. In the study of Buchanan and Lovallo (2001), it was found that the administration of cortisol (20 mg) 1 h prior to learning enhanced long-term memory for relatively arousing pictures but not for relatively neutral pictures. Another study showed that postlearning stress hormone activity, namely cortisol increase, was associated with enhanced memory for emotionally arousing slides but not for relatively neutral slides (Cahill et al. 2003). Besides the well-described effects of glucocorticoids on the acquisition and consolidation of emotional memory, glucocorticoids also affect the memory retrieval mechanism. There is evidence for the preventive effects of cortisol against PTSD. For example, prolonged administration of cortisol (100 mg/day for 6 days) during intensive care treatment reduced the risk of later occurrence of PTSD, possibly by inhibiting excessive retrieval of traumatic memories (Schelling 2002; Schelling et al. 2001). Furthermore, a recent pilot trial using a double blind, placebo-controlled, crossover design revealed that cortisol treatment (10 mg/day for 1 month) reduced the intrusive and avoidance symptoms in chronic PTSD patients (Aerni et al. 2004).

The hippocampus is a brain structure involved in learning and memory, especially declarative memory (Elzinga and Bremner 2002). It has been suggested that the hippocampus plays a role by itself in memory consolidation in neutral circumstances, but that in circumstances of emotional arousal, the amygdala boosts this hippocampal function. This structure is a neural glucocorticoid (GC) target site, with an abundance of GC receptors (Sapolsky 2000). After an initial increase in cortisol release, a negative feedback system is activated to keep cortisol levels within the physiological range, which is regulated primarily by the hippocampus. GC receptor activation in the hippocampus has been suggested to underlie the effects of cortisol on memory in humans (Lupien and Lepage 2001). In addition, it has been suggested that prolonged stress or prolonged exposure to glucocorticoids can have adverse effects on the hippocampus, and PTSD has been reported to be associated with a hippocampal volume loss (Sapolsky 2000).

4.3. Neuroimaging Studies in Cancer-Related Intrusion

Attention is currently being focused on the volume of the limbic structures, as well as their functions in memory consolidation. Previous structural magnetic resonance imaging (MRI) studies in PTSD have revealed that chronic PTSD is associated with lower hippocampal volume in adult patients having PTSD related to combat exposure, childhood sexual and physical abuse, or miscellaneous trauma (Bremner et al. 1995; Bremner et al. 1997; Gilbertson et al. 2002; Gurvits et al. 1996; Stein et al. 1997; Villarreal et al. 2002). In addition, reduced total white-matter volume (Villarreal et al. 2002) and reduced anterior cingulate volume (Yamasue et al. 2003) have

also been reported.

On the other hand, little is known about the volumes of the hippocampus and amygdala in persons with cancer-related intrusion. There is only one study that reported the volumes of these structures in breast cancer survivors with or without intrusive recollection (Matsuoka et al. 2003; Nakano et al. 2002); the study showed that women with a history of cancer-related intrusive recollections showed a 4.9% smaller left hippocampal volume and 5.7% smaller left amygdala volume as compared with those without such history (Matsuoka et al. 2003; Nakano et al. 2002). Furthermore, women with a history of cancer-related intrusive recollections showed impaired visual declarative memory as compared with those without such a history (Nakano et al. 2002). However, the fundamental questions of whether such volumetric differences represent the neurotoxic effect of persistent intrusive recollections for several years or a preexisting trait that predisposes people to pathological stress reactions to traumatic cancer experiences still remain to be answered.

5. Conclusion

There are still many unanswered questions pertaining to cancer-related intrusion. Initial data suggest that a significant proportion of cancer survivors suffer from intrusions, which emphasizes the need for more comprehensive longitudinal studies. Specifically, more qualitative studies to determine the distinctive features of cancer-related intrusion, functional neuroimaging studies to elucidate its neurobiological basis, and genetic and molecular biological studies to identify vulnerable individuals are indicated. The present authors believe that women with cancer-related intrusion display abnormal functional connectivity involving the amygdala, hippocampus, and prefrontal cortex, and that these patterns are amenable to modification by pharmacological interventions such as administration of β -adrenergic blockers and agents that regulate cortisol levels. Studies may also be required to assess the potential buffering effect of high-quality communication on the traumatizing impact of the disclosure of cancer diagnosis, either at the initial diagnosis or following relapse diagnosis (Ronson 2004).

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Earthquake-Related PTSD: A Follow-up Study in Prevalence, Comorbidity, Quality of Life, and Biological Correlates

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1. Introduction

Disasters, both natural and man made, affect millions of people around the world each year. Breslau et al. (1991) estimated that 6%–7% of the US population was exposed to disaster or physical or psychological trauma every year. Wang et al. (2000) showed that natural disasters affect an average of approximately 200 million people in China each year, killing several thousands. Two of the most recent and devastating traumatic events were the 9/11 terrorism attack that occurred in the USA in 2001 and the tsunami disaster in South Asia in 2004. Most studies have found evidence of psychiatric disorders among residents of affected areas after disasters, including posttraumatic stress disorder (PTSD), major depressive episode (MDE), sleep and anxiety disorders, and substance abuse (Goenjian et al. 2000; Green et al. 1992; Maj et al. 1989; McFarlane and Papay 1992; Rubonis and Bickman 1991). MDE and PTSD are the most common disaster-related psychiatric diagnoses, and are strongly associated with each other (Chang et al. 2003; Chou et al. 2003; Goenjian et al. 2000; Green et al. 1992; McFarlane and Papay 1992). The reported PTSD incidence in various studies has ranged from 6%–74% (Breslau et al. 1991; Carr et al. 1995; Chang et al. 2002, 2003; Chen et al. 2001a; Chou et al. 2003; Hsu et al. 2002; Kuo et al. 2003; Tainaka et al. 1998; Papadatos et al. 1990; Yang et al. 2003), occurring predominantly in women (Chang et al. 2003; Chou et al. 2003). PTSD can be triggered by a variety of traumatic events (e.g., earthquake; EQ) and has been found to be associated with the development of all other psychiatric disor-

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ders examined (Brady et al. 2000; Chou et al. 2003; Goenjian et al. 2000). However, Krause (1987) argued that careful consideration must be given to the time interval between the occurrence of a stressful situation and data acquisition, as well as the length of time that is required for symptoms to abate. The meaning attributed to a traumatic event is the product of a complex interaction that is based on the event itself, personal histories and future expectations, and biological factors, all combining to produce the psychiatric impairment or disorder (Ursano et al. 1992).

1.1. Impact of Chi-Chi Earthquake on Taiwan

A devastating earthquake registering 7.3 on the Richter scale struck Taiwan in the early morning of 21 September 1999. According to the official report, the Chi-Chi earthquake (so named because the epicenter was near Chi-Chi) resulted in the deaths of 2328 people with 8722 injured and more than 4000 homes destroyed. As a consequence of the EQ, its survivors also suffered from severe psychological trauma and mental disorders, catching the attention of all in society. Within 1 month of the EQ, Chen et al. (2001a) found that 90% of a population of 525 surviving victims registered in a rescue working station appeared to have psychiatric morbidities such as insomnia, psychological stress, nervousness and anxiety, low mood, dizziness and headaches, chest distress, and acute posttraumatic stress syndrome. Among those suffering from this acute stress syndrome, the repeated occurrence of an EQ image was reported by 66%, avoidance and numbness by 20%, and insomnia and startling by more than 60%. The Department of Health of Taiwan not only attended to the immediate physical injuries during the initial stages after the EQ, but it also set as a priority the mental well-being of those involved in the EQ, citing the importance to the survivors of psychological reconstruction. Subsequently, the Department requested all the psychiatrists on the island of Taiwan to join in the effort of psychological reconstruction. Most of the mental health workers were trained and were assigned responsibility for 25 affected areas 6 months after the EQ. The goals of this project were to screen victims who were psychologically or mentally ill as a result of this natural disaster and to initiate interventions. Eighteen study reports related to the Chi-Chi earthquake (Table 1) were published by the end of 2004 (Chou et al. 2005b). These included: (1) development of screening instruments for PTSD and MDE, (2) identification of risk factors for PTSD and subsyndromal PTSD, and (3) measurement of quality of life among survivors. The study subjects included not only survivors of all ages, but also rescue workers and relatives of the deceased.

This chapter illustrates the psychological morbidity-related work of one of the main research teams, particularly with regard to PTSD. Its focus includes prevalence, comorbidity, quality of life, and biological correlates. This research work should also provide important guidelines for responding to future disasters.

2. Methodology

This two-stage epidemiological study was conducted during two periods: around 4–6 months and 20–36 months following the disaster. Stage 1 was a questionnaire survey of psychiatric disorders in the Yu-Chi township close to the Chi-Chi area. Stage 2 was conducted at Tong-Chi village, part of the Yu-Chi township, to further validate the psychiatric diagnoses. Residents of the former location ($n = 4223$) were administered the questionnaire “Disaster-Related Psychological Screening Test (DRPST)” (Chou et al. 2003); the adjusted response rate was 60.3%. Residents of the latter location ($n = 461$) were administered both the DRPST and the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al. 1998) in person to determine their psychiatric diagnoses; the adjusted response rate was 79.9% at the first time point (4–6 months). Similar response rates were found at the second time point for both study populations. Demographic information in both populations showed slightly advanced age (mean 51 ± 18.1 years) and low education level (more than 51% were primary school educated or below), but with an even number of men and women.

2.1. Establishment of the Disaster-Related Psychological Screening Test

To create a short screening scale for the detection of PTSD and MDE, DRPST was designed to collect background information on residents and check for psychological symptoms resulting from disaster-related psychiatric disorders (including 17 items for PTSD and 9 items for MDE) according to DSM-IV criteria. DRPST and MINI interview were both administered to Tong-Chi village population ($n = 461$). Logistic regression analysis of predictors for MINI diagnosed-PTSD and MDE revealed that 7 of 17 PTSD symptoms and 3 of 9 MDE symptoms from the DRPST had overall predictive capabilities of 98.3% and 97.6%, respectively (Chou et al. 2003). Scores of 3 or more on the 7-symptom PTSD scale and 2 or more on the 3-symptom MDE scale for cut-off points were used to define a group of positive cases. Validated against the MINI as the gold standard, the sensitivity and specificity of this short screening scale for PTSD were 97.8% and 96.6%, respectively; for MDE they were 92.1% and 98.3%, respectively. Positive predictive value for PTSD was 76.3% and for MDE was 83.3%. The DRPST, which was administered during phase 1 of the two-phase study, may be used for effective and rapid screening for PTSD and MDE after natural disasters such as earthquakes.

Table 1. Summary of Chi-Chi earthquake articles related to psychiatry (Medline search through 2004)

Authors	Year published	Study period after earthquake	Subjects	Purpose	Method	Conclusion
Chen et al.	2001	Within 1 month	525 residents	Screening for psychiatric morbidity and posttraumatic symptoms among survivors in the early stage	Purposeful sampling	11% of the subjects reported having thought of death or having suicidal ideation; 89.9% of the respondents had psychological impairment
Chen et al.	2001	Within 1 year	210 residents	The Chinese version of the Davidson Trauma Scale: a practice test for validation	Back-transback translation and concurrent validity	Sensitivity of the instrument was 0.9, specificity 0.81, positive likelihood ratio 4.74, and negative likelihood ratio 0.12
Hsu et al.	2002	Six weeks later	323 student residents	Posttraumatic stress disorder among adolescent earthquake victims in Taiwan	Purposeful sampling	21.7% of 323 students demonstrated PTSD. Being physically injured and experiencing the death of a close family member with whom they had lived were the two major risk factors for PTSD
Shih et al.	2002	Within 1 year	46 nurses who worked in a hospital in the community	The impact of the 9-21 earthquake, experiences of Taiwanese nurses as rescuers	Purposeful sampling	Rescue experiences help to strengthen most Taiwanese nurses' professional competency
Liao et al.	2002	Two months later	1104 rescue workers serving in the earthquake	Association of psychological distress with psychological factors in rescue workers	Purposeful sampling	Prevalence of general psychological distress is high among rescue workers. Personality traits and pre-disaster life adjustment had a dominant predictive power for psychological distress
Lin et al.	2002	One year later	368 residents, with 268 aged 65 years or older	Geriatric survivors	Purposeful sampling	Poor quality of life
Chang et al.	2002	Six months later	171 pregnant residents	Psychiatric morbidity and pregnancy outcome in a disaster area	Purposeful sampling	The prevalence of minor psychiatric morbidity (MPM) was 29.2%
Yeh et al.	2002	Within 16 days	187 young, male military personnel who served as rescue workers	Characteristics of acute stress symptoms and nitric oxide concentration in young rescue workers in Taiwan	Purposeful sampling	Significant inverse correlation was observed between severity of stress symptoms and the plasma concentration of nitric oxide in the rescue worker group
Chou et al.	2003	Twenty-one months later	461 residents	Establishment of a disaster-related psychological screening test	Population survey	The DRPST, which was administered for phase 1 of this two-phase study, may be used for effective and rapid screening for PTSD and MDE after an earthquake
Chang et al.	2003	Five months later	84 male firefighters	Posttraumatic distress and coping strategies among rescue workers	Purposeful sampling	16.7% and 21.4% prevalence of general psychiatric morbidity and posttraumatic morbidity

Kuo et al.	2003	One year later	120 residents	Prevalence of psychiatric disorders among bereaved survivors	Purposeful sampling	The two most prevalent disorders were PTSD (37 %) and major depressive disorder (16 %)
Chou et al.	2003	Two to 15 months after the earthquake	3 432 705 residents	Suicides after the 1999 Taiwan earthquake	Population cohort study	Mental health programmes or other preventive strategies might be more effective by specifically targeting victims rather than by simply targeting individuals living in earthquake-affected areas
Yang et al.	2003	Three months later	663 residents	Psychiatric morbidity and posttraumatic symptoms among earthquake victims in primary care clinics	Purposeful sampling	The rate of posttraumatic stress disorder was 11.3%, and the rate of partial PTSD was 32.0%
Guo et al.	2004	252 rescue workers	252 rescue workers	Posttraumatic stress disorder among professional and nonprofessional rescuers	Purposeful sampling	Disaster rescue work is associated with a high level of stress even for highly trained professionals and may lead to mental health problems.
Chou et al.	2004	Six months after the earthquake	4223 respondents	The relationship between quality of life and psychiatric impairment for a Taiwanese community postearthquake	Population survey	The greater the severity of the psychiatric impairment, the lower the scores for quality of life, for both the physical and mental aspects of this important general indicator
Chou et al.	2004	Twenty-one months after the earthquake.	461 respondents	Quality of life and related risk factors in a Taiwanese village population	Population survey	The earthquake survivors had a higher percentage of psychiatric disorders. The risk factors that affected quality of life in survivors were age, female, financial loss, social network change, and mental impairment
Lai et al.	2004	Ten months after the earthquake	Subjects ($n = 252$) randomly selected from two rural communities	Full and partial PTSD among earthquake survivors in rural Taiwan	Population survey	PTSD and PTSS are commonly observed following earthquake exposure and are associated with similarly high levels of psychosocial impairment
Liao et al.	2004	Eighteen months later	92 subjects who visited emergency medical stations immediately after the giant earthquake	Hyperleptinemia in subjects with persistent partial posttraumatic stress disorder after a major earthquake	Purposeful sampling	Subjects with persistent subsyndromal posttraumatic stress disorder and hyperarousal had significantly higher serum leptin levels

Resources of all the published articles are listed in the References Section

3. Main Findings

3.1. *Prevalence of Psychiatric Morbidity*

Based upon the DRPST, at 4–6 months after the EQ, the Yu-Chi township population ($n = 4223$) was found to have the following prevalences: PTSD 21.3%, MDE 15.1%. In the population of Tong-Chi village ($n = 461$) (Chou et al. 2005a), based upon the MINI, these estimates were two- to threefold lower than in the former population: prevalences were PTSD 7.9%, MDE 9.5%, and panic disorder 1.6%. At the second data acquisition time, a three- to sevenfold reduction in prevalence of the above mental disorders for the larger Yu-Chi population was observed whereas there was only about a twofold decrease in PTSD (Chou et al. 2004a) in the smaller Tong-Chi population. In a fixed cohort of the Tong-Chi group ($n = 216$), between the first and second time points, despite the twofold decrease for PTSD and MDE, dysthymia remained at the same rate, around 5%, and the suicide rate increased from 4.2% to 6.0% (unpublished data). The increase in the suicide rate might be related to dysthymia, which was attributed to lack of a social network. Twenty-three cases of delayed-type PTSD that occurred 18 months after the EQ were also found in this population.

3.2. *Comorbidity of PTSD*

At 6 months after the EQ, cases of PTSD at Tong-Chi village had greater concurrent psychiatric disorders according to the MINI than non-PTSD cases with the odds ratios being: panic disorder 17.4, MDE (current) 14.4, and dysthymia 2.8. There was no increase in the risk of alcohol and drug dependence (Chou et al. 2005a). However, at the second time point, PTSD subjects had greater risk of concurrent MDE (odds ratio 14.8) and alcohol abuse (odds ratio 4.6) than did non-PTSD cases. It appears that PTSD is often comorbid with major depression in the aftermath of, or a long time after, an EQ.

3.3. *Clinical Course of PTSD, MDE, and PTSD Comorbid with MDE*

Table 2 presents changes of the clinical courses of PTSD, MDE, and PTSD combined with MDE, based upon the DRPST, across a 2.5-year period in a fixed cohort of the Yu-Chi study population ($n = 1756$). At the second follow-up, around 60% of the above three groups were found to have no significant mental disorders. Of the subjects in the PTSD or MDE or PTSD plus MDE categories in 2000, upon follow-up in 2002, 10%–13% met the criteria for MDE, which was twofold the number of subjects who met the criteria for PTSD (5%–6%) and fourfold the number of sub-

Table 2. Clinical courses of psychiatric cases from years 2000–2002 in a fixed cohort of Yu-Chi township ($n = 1756$)

Year 2002	Year 2000								
	MDE			PTSD			PTSD + MDE		
	$(n = 294)$			$(n = 408)$			$(n = 159)$		
	n	(%)	P value	n	(%)	P value	n	(%)	P value
MDE	38	(13%)	<0.001	39	(10%)	<0.01	17	(11%)	<0.05
PTSD	16	(5%)	<0.05	20	(5%)	<0.1	10	(6%)	<0.05
PTSD + MDE	7	(2%)	NS	12	(3%)	<0.01	6	(4%)	<0.05

PTSD, posttraumatic stress disorder; MDE, major depression episode

jects who met the criteria for PTSD comorbid with MDE. These data suggest that, in 2000, MDE was just as good a predictor as PTSD for PTSD in 2002. Similarly, in 2000, PTSD was nearly as good a predictor as MDE for MDE in 2002. In other words, there is a high level of crossover of these two disorders over time. In addition, PTSD comorbid with MDE in 2000 was a good predictor for either MDE or PTSD in 2002. This follow-up observation also indicated that the combined condition found earlier after the EQ might have separate evolution into PTSD or MDE after a long-term course.

3.4. Assessment of Quality of Life in PTSD

To investigate the relationship between quality of life and related factors by comparing post-EQ survivors stratified according to degree of psychiatric impairment, the 36-item Short-Form Healthy Survey (SF-36) (Hays et al. 1988) and DRPST (Chou et al. 2003) were administered to the Yu-Chi population at 4–6 months (Chou et al. 2004b). Twelve of 17 of the items of the DSM-IV PTSD symptoms list and 7 of 9 items of DSM-IV MDE symptoms were selected for the DRPST after validation. Table 3 lists the frequency of these PTSD or MDE symptoms reported by 4223 subjects of the Yu-Chi population. The score range for the DRPST is 0–19 (PTSD 12; MDE 7). The population was divided into four psychiatric impairment groups according to DRPST scores: (1) healthy (no PTSD or MDE symptoms); (2) mild (1–6); (3) moderate (7–12); and (4) severe (13–19). Psychiatric impairment that was rated as moderate to severe was reported by 34.3% of the responding residents. For all SF-36 subscale scores, including four physical and four emotional component summaries, the greater the severity of the psychiatric impairment, the lower the scores for quality of life for both the physical and mental aspects of this important general indicator (all P values <0.001). These results further support the hypothesis of Cass et al. (1999) that quality of life may be used to evaluate mental-health outcome. In addition, these psychiatrically impaired respondents tended to be older, female, divorced or widowed, less educated, and with major financial losses immediately after the EQ. These risk factors also affected the quality of life of the survivors.

Further assessment of the relationship between quality of life and psychiatric disorders in the Tong-Chi village survivors 21 months after EQ revealed poorer quality of life in the order of PTSD combined with MDE > MDE > PTSD > other

Table 3. Symptoms of PTSD and MDE as reported by 4223 subjects at Yu-Chi township

Symptoms of PTSD	<i>n</i>	%	Symptoms of MDE	<i>n</i>	%
Reexperiencing			Core symptoms		
1. Intrusive and distressing recollection, thoughts	1100	26.0	1. Depressed mood most of the day, nearly every day.	1646	39.0
2. Distressing dreams of the trauma	634	15.0	2. Marked diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day	1161	27.5
Avoidance and numbing			Associated symptoms		
1. Diminished interest in activities	1161	27.5	1. Diminished ability to think or concentrate or indecisiveness, nearly every day	1546	36.6
2. Efforts to avoid thoughts, feelings associated with the trauma	590	14.0	2. Insomnia or hypersomnia nearly every day	1398	33.1
3. Sense of foreshortened future	511	12.1	3. Psychomotor agitation or retardation nearly every day	1159	27.4
4. Efforts to avoid activities, places, people that arouse recollections of the trauma	471	11.2	4. Fatigue or loss of energy nearly every day	551	13.0
5. Feeling of detachment from others	288	6.8	5. Feeling of worthlessness or excessive or inappropriate guilt nearly every day	511	12.1
Arousal					
1. Exaggerated startle response	2163	51.2			
2. Hypervigilance	1879	44.5			
3. Difficulty concentrating	1546	36.6			
4. Difficulty falling asleep or staying asleep	1398	33.1			
5. Irritability, outbursts of anger	1159	27.4			

psychiatric diseases > mentally healthy groups on both the physical or mental aspects of the SF-36 (Chou et al. 2004a). Although almost 2 years had passed, the risk factors for the poorer quality of life were still elderly, female, financial loss immediately after EQ, lack of social network, and mental impairments, particularly PTSD, MDE, and sleep disturbance, suggesting that these factors may exert a long-term impact on the quality of life of the surviving EQ victims.

3.5. Biological Correlates of EQ-Related PTSD

With the hypothalamic-pituitary-adrenocortical axis (HPA) having been widely studied from bench to clinic under stress-related conditions, we studied this system in our EQ-related PTSD population. Salivary cortisol from baseline and after the dexamethasone suppression test (DST) was collected 1.5 years after the EQ in three groups of subjects: PTSD subjects, non-PTSD subjects of the disaster area, and control subjects far away from the disaster area. The EQ appeared to modulate cortisol levels. PTSD subjects had lower basal cortisol levels than both groups of non-PTSD subjects. PTSD and non-PTSD subjects in the disaster area had less suppression of cortisol following the DST than control subjects (Su et al. 2001). Lower cortisol levels in PTSD subjects was consistent with (1) the lower plasma cortisol levels of adolescents who had high exposure to the 1988 earthquake in Armenia, 5 years into the aftermath (Goenjian et al. 1996); and with (2) low urinary cortisol secretion of Holocaust survivors with chronic PTSD (Yehuda et al. 1995a). Oquendo et al. (2003) also found a similar result for PTSD comorbid with MDE, highlighting the importance of considering comorbidity in psychobiology. Additionally, Yehuda's finding of enhanced cortisol suppression in response to the low-dose dexamethasone suppression test (DST) in combat veterans (Yehuda et al. 1993, 1995b) has been replicated in adult survivors of childhood sexual abuse (Stein et al. 1997), children exposed to natural disasters (Goenjian et al. 1996), and Desert Storm returnees (Kellner et al. 1997). On the contrary, survivors of the EQ in our study, either PTSD or non-PTSD, had less suppression of cortisol on the DST than did control groups. These different results might be explained by different durations from the onset of trauma (1.5–3 years for our study vs. >5 years for the others) and by different etiology of stress (earthquake vs. combat or sexual or childhood abuse). Whatever the findings were for PTSD subjects, the functioning of the HPA axis was certainly altered. We also found that PTSD subjects had worse attention and memory when compared with non-PTSD victims and far-removed control subjects on neurocognitive assessment (Su et al. 2001). This is consistent with the report of Gilbertson et al. (2001), which states that attention and memory tasks provide unique and independent prediction of PTSD versus non-PTSD status in combat veterans. Follow-up studies of our PTSD subjects were conducted 2 years later, after the first trial, and revealed partial recovery in both HPA axis function and neurocognition (Su et al. 2004), suggesting that subjects influenced by EQ stress were still affected.

4. Conclusion

Our study provides a model of a two-stage investigation for epidemiological surveys in areas affected by natural disasters. First, we developed a questionnaire for screening mental disorders, particularly PTSD and MDE that included both a cross-sectional survey (within 6 months) and a follow-up study yearly for 3–5 years. Our data show that a major earthquake may induce severe stress and result in long-term bio-psycho-social effects on subjects. However, the prevalence of PTSD, MDE, and panic disorder decrease over time, while quality of life improves. Despite that, our study also found partial recovery of the HPA axis function, reflected by low baseline cortisol level and deficits in DST results, and abnormal neurocognitive function. Persistent abnormalities in these biological aspects, even a long time after the disaster had passed, may result in some of the survivors still being affected by stresses such as family support system, financial stress, and degree of property damage. During the natural disaster, being elderly, female, widowed or divorced, having little education, and having major financial losses immediately after the disaster are risk factors for the occurrence of mental disorders, and are predictors for low quality of life. The greater the psychiatric impairment, the worse that the quality of life becomes. Also, quality of life may be used to evaluate mental health outcomes. Biological deficits induced by catastrophic trauma should not be neglected, and indicate the necessity of early biological and pharmacological intervention. Finally, because the etiology of PTSD may be linked to natural disasters, infectious diseases, and terrorism, PTSD may become more heterogeneous. Thus, studies of clinical aspects, neuroendocrinology, and brain mechanisms in PTSD are desperately needed.

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Psychological Consequences for Students Who Survived the Ehime Maru Accident: A 26-Month Follow-Up Study

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1. Introduction

In recent years, many studies have investigated the psychological effects of transportation disasters on survivors and the bereaved. In particular, these reports have examined cases of catastrophic maritime, rail, and air accidents in order to establish effective interventions for improving the outcomes of victims. Joseph et al. (1994, 1997) and Dalglish et al. (1996) have described the Herald of Free Enterprise car ferry disaster that occurred in Zeebrugge, Belgium. In that accident, 193 passengers died and 351 survived. These studies (Joseph et al. 1994; Dalglish et al. 1996) showed that greater crisis support was correlated with lower levels of avoidance and depression. Furthermore, Joseph et al. (1992) reported in a study of the survivors of the Jupiter cruise ship disaster that higher levels of crisis support were strongly related to better psychological outcome. Although the aforementioned studies emphasize the importance of mental support for the survivors of maritime accidents, few studies have described such support in detail.

In contrast to other sea accidents, the Ehime Maru sea disaster included unique characteristics. First, this accident was caused by a collision between a US nuclear submarine and a Japanese training vessel. Second, this accident caused serious political problems between Japan and the USA. Third, not only crewmembers of the vessel but also many high school students died, giving the Ehime Maru accident the characteristics of a school disaster as well as a transportation disaster. As we began to examine the mental states of the survivors in order to provide interventions for them, we encountered many difficulties arising from this dual nature of the disaster. However, over time these problems were overcome.

To demonstrate the psychological influence of the accident and the effectiveness of the intervention provided to the survivors, this chapter details the mental states of the students who survived the accident and provides data on the incidence and long-term outcome of posttraumatic stress disorder (PTSD) and depression.

2. The Accident

The Ehime Maru, a Japanese training vessel, was hit by the US submarine *Green-ville* about 18 km off Honolulu, Hawaii, on 9 February 2001. Soon after the collision, the Ehime Maru sank quickly with 35 people on board. The Coast Guard rescued 26 people; however, 4 students, 2 teachers, and 3 crewmembers were missing and presumed dead. The accident occurred during a training voyage for students of the Uwajima Fisheries High School in Uwajima City. Afterward, the US Navy officially acknowledged negligence on the part of the submarine crew and apologized to the victims and Japanese government.

3. Mental Care and Support Provided to Students

Soon after the accident, a mental support team consisting of several psychiatrists and clinical psychologists was organized in order to assess posttraumatic responses among the students who survived the accident and to provide psychiatric care for them. The team provided suggestions to teachers and local team members who belonged to the health center and psychiatric hospital in Uwajima. The following support programs were provided for the students:

1. Visiting services for students and families were regularly provided by district nurses.
2. Daycare, designed specifically for the surviving students, was carried out in order to establish normal circadian rhythms among the students and to facilitate various self-help activities.
3. Group meetings were held for students' families once a week for approximately 2 years after the accident.
4. After graduation, the local employment agency assisted many students in finding employment.

In addition to the above programs, the media attention on Uwajima was so intense that the mental support team frequently demanded that media outlets refrain from conducting excessive interviews. However, the team was able to capitalize on this media attention by informing the residents of Uwajima about the posttraumatic responses of the students through the media.

4. Subjects and Methods

All nine students who survived the accident were examined by the members of the mental support team at 2, 8, and 14 months after the accident. At the time of the accident, all subjects were boys in their second year of high school. Prior to the accident, most of the students had hoped to become fishermen. At each examination,

the students completed three self-rating scales: the Japanese-language version of the Impact of Event Scale-Revised (IES-R) (Asukai 2002), the General Health Questionnaire 28 items (GHQ28) (Goldberg and Hillier 1979) and the Self-rating Depression Scales (SDS) (Zung et al. 1965). All subjects were assessed and diagnosed using not only standard psychiatric interviews, but also using the Clinician-Administered PTSD Scale (CAPS; Blake et al. 1995), which is regarded as a practical and reliable structured interview for PTSD (Weathers et al. 2001).

5. Results

5.1. Self-Rating Scales

At 2 months after the accident, the mean scores on all the self-rating scales were very high, viz., 51.2 on the IES-R, 13.1 on the GHQ, and 51.5 on the SDS (Fig. 1), exceeding the PTSD cutoff points of 24/25 for the IES-R total score, 5/6 for the GHQ score, and 47/48 for the SDS obtained from previous research in Japan (Asukai 2002, Fukunishi 1990). Mean scores of the students were greater than these cutoff points until 8 months after the accident. Although these scores remained relatively high until the 14-month examination, they normalized at 26 months after the accident.

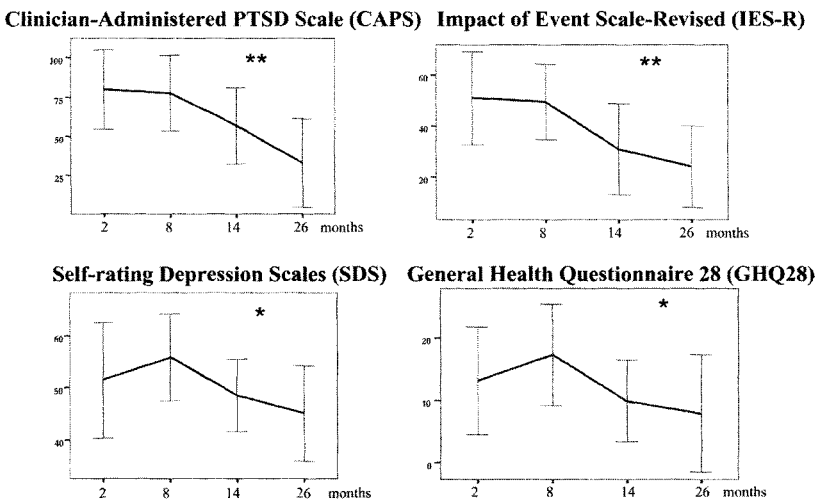


Fig. 1. Changes in mean scores of Clinician-Administered PTSD Scale (CAPS), Impact of Event Scale-Revised (IESR), Self-rating Depression Scales (SDS), and General Health Questionnaire 28 (GHQ28) at 2, 8, 14, and 26 months after the accident. Data was analyzed using Friedman's test for repeated measures. PTSD, posttraumatic stress disorder. Asterisk, $P < 0.05$; double asterisk, $P < 0.01$

5.2. CAPS and Standard Psychiatric Interviews

According to the results of the CAPS and standard psychiatric interviews at 2, 8, and 14 months after the accident, most students were diagnosed with full PTSD and major depression (Table 1). During the interviews, as the students spoke of their experiences during the accident, it became clear that most of them were suffering from serious PTSD symptoms. For example, at the time the Ehime Maru sank, the inside of the vessel suddenly became dark. Thereafter, many of the students developed a fear of darkness and kept their lights on throughout the night. This resulted in serious sleep disturbance and a reversed circadian rhythm, which prevented them from going to school and led to extended periods of social withdrawal.

In addition to these PTSD symptoms, most students felt intense survivor guilt that developed into a profound grief reaction and major depression. Students blamed themselves for failing to help their classmates and teachers during the accident. For example, students said “If I had cried more loudly at that time, he could be alive,” or “I was confused, so I didn’t go back to the ship to help our teachers. I should have done...” Moreover, most of the students were unable to believe that their classmates and teachers were dead for several months after the accident and were unwilling to attend any funerals.

Shortly after the 8-month examination, the Ehime Maru was raised and transported closer to Oahu, where navy divers were able to recover most of the victims’ bodies. Most students became confused and depressed when forced to confront the fact that their classmates would never return to their hometown. Three students who indicated during our interviews that they were experiencing suicidal ideation were immediately admitted to a psychiatric hospital in order to prevent any suicide attempts.

Table 1. Diagnoses among students (n=9)

	PTSD		MDE
	full	partial	
2 months	7	2	6
8 months	8	1	6
14 months	7	1	4
26 months	1	4	1

Finally, many students were able to attend the ceremony for the first anniversary of the accident, and gradually began to accept their classmates' deaths. Although the students' psychiatric states were still generally poor, at the 14-month examination they appeared to be beginning to release themselves from their survivor guilt. Students who were able to graduate on schedule gradually emerged from their state of social withdrawal and began to seek employment or begin further education. At the conclusion of this 26-month study, only one student was diagnosed with full PTSD, with major depression also noted for the same individual. Over time, many of the students began to regard the accident as a tragedy that none of them were responsible for. They were able to have hope for the future and found employment in various places such as a fish processing factory or a restaurant.

6. Discussion

6.1. Consideration of Mental Status of Students at the First Two Examinations

The general features of the mental status of the students at the first two examinations were as follows:

1. The prevalence of PTSD was at a very high level of approximately 89%. In previous studies of transportation disasters, there have rarely been occurrences of PTSD with such a high rate of frequency. To our knowledge, the highest rate of frequency in past studies of maritime accidents was 51.5 %, which was obtained using the CAPS, 5 months after the sinking of the Jupiter in Greek waters (Yule et al. 2000). In consideration of the fact that such a high prevalence rate was not observed among the crew, this is regarded as a phenomenon specific to the surviving students.
2. Major depressive episodes occurred concurrently with PTSD at a high rate of frequency, and the CAPS total scores were also strongly correlated with the SDS scores ($r = 0.852$, $P < 0.01$). Most students suffered from survivor guilt and profound grief reactions and therefore felt shame in living longer. The members of the mental support team were afraid that some of the students might attempt suicide, especially when the ship was raised.
3. Many of the students had serious sleep disturbance and a reversed circadian rhythm, which led to a state of social withdrawal. The existence of such intrusive symptoms, in addition to arousal symptoms, is also the cause believed to be responsible for the serious sleeping difficulties. This sleep disturbance was one of the major factors that prevented the surviving students from returning to school.

As mentioned above, the surviving students all displayed serious mental statuses. We consider below the causes on the two points of a high prevalence of PTSD and a delay of recovery.

6.1.1. High Prevalence Rates of PTSD and Depression

In general, the PTSD prevalence rates reported in transportation disasters have been obtained from investigations conducted on passengers. For example, the results of a major mental health examination of the 79 surviving passengers of the Boeing 737 crash in Kegworth, England, in 1989 revealed that 40% of those passengers had PTSD and that 33% met the diagnostic criteria for major depression in a follow-up study 1 year after the accident (Gregg et al. 1996). Although many similar studies have examined the magnitude of the effects of a transportation disaster on the mental health of passengers, the results of the first two examinations of the students showed an almost unprecedented PTSD prevalence rate.

Furthermore, as another major feature of the Ehime Maru accident, symptomatic differences were observed between the students and the crew. Namely, with respect to PTSD prevalence rates and other scale scores, symptoms among the crew were less serious than those of the students, making it almost appear as if the crewmembers were victims of a different accident. Therefore, it is necessary to consider the psychopathology and group dynamics of the surviving students in order to explain the difference between these two groups.

Cohesiveness Among the Students

The students who participated in the navigational training aboard the Ehime Maru were classmates who had been studying in the same class for approximately 2 years since enrolling in the Uwajima Marine and Fisheries High School. In addition, prior to the accident, the students had arrived in Hawaii after completing a month-long voyage without calling at any other port since setting sail. During this period, while receiving dedicated guidance and training from the teachers and the crew, the students presumably heightened their team awareness and sense of belonging amid the harsh conditions of the Pacific Ocean in the winter. Being shut off from the outside world in this way facilitated the development of a highly cohesive group. Notably, the students were in their adolescence, which is a developmental period when the creation of a same-sex peer group with strong feelings of belonging is important to developing a sense of self-identity (Erikson 1959). Such idiosyncrasies of adolescence also contributed to the fostering of a stronger sense of cohesiveness than among the crew.

During examinations and interviews for the present research, many students strongly blamed themselves for their behaviors at the time the vessel sank and had strong survivor guilt, asking themselves, "If I made a greater effort, could I have saved my friends?" Many students fell into a depressive state and experienced suicidal ideation. The appearance of such a strong grief reaction arose from the strong sense of cohesiveness that developed between the students. Thus, this sense of cohesiveness is considered to have played an important part in causing the high PTSD incidence rate and the strong grief reactions.

Sloan (1988) examined posttraumatic stress in 30 young male survivors of an airplane crash-landing who belonged to the same college basketball team. Sloan found that the survivors initially experienced high levels of stress, which decreased

rapidly and stabilized over time. Although this result suggests that cohesiveness among young adults may result in positive mental health outcomes, there is a great difference between this aircraft accident and the Ehime Maru accident. The accident examined by Sloan was not fatal. van der Kolk (1985) has noted the unique vulnerability to PTSD among young soldiers serving in the Vietnam War; the young survivors of the Ehime Maru accident likely displayed reactions similar to combat veterans rather than passengers.

Preparedness for an accident

One of the greatest differences between the surviving students and the crew lies in their preparedness for an accident. The crewmembers had cumulative navigating experience, actual experience in dangerous situations, and were prepared for an accident. In contrast, although they had received emergency evacuation training, the students had never experienced an accident until the present disastrous voyage. It can be conjectured that this lack of experience and mental preparation resulted in psychological trauma that was worse than among the crew. The uniqueness of the accident itself, namely the extraordinary circumstances in which a massive submarine suddenly collided with the bottom of their ship causing it to sink in only minutes, likely far surpassed the worst situation the students could have imagined.

6.1.2. Prolonged Posttraumatic Responses

Another major characteristic concerning the mental status of the surviving students was that little or no change was observed for approximately 1 year after the accident. Many of the students were unable to even leave their homes and lived continuously inside their houses for many months. Although this delay in the recovery of symptoms may have been unavoidable in view of the severity of the posttraumatic responses caused by this accident, several other factors may have been responsible for the prolonged posttraumatic responses.

Crisis of Vocational Identity

The PTSD symptoms that appeared in the students were unbearable for them. However, what made the situation more serious was the fact that this accident occurred while they were undergoing navigational training, not during leisure activities. Before the accident occurred, the intention of most of the students was to become employed in fishery-related work after graduation or to enroll in a more specific professional school in order to become higher-class seamen. The students' parents were mostly involved with fisheries, and a majority of the students had dreamed of becoming seamen since childhood. One of them commented, "Ever since I can remember, I have never thought of doing work other than that of a seaman." Many students even indicated that they enrolled in the Uwajima Marine and Fisheries High School solely because the school had a navigational training course, no matter how rigorous it might be. The PTSD symptoms brought on by this accident deprived them of such hopes for the future. For a long time, many of them were at a loss as to how they should live their lives.

Delayed Grief Work

In this accident, the missing persons remained deep under the sea, and their deaths could not be confirmed until the submerged ship was raised in October 2001. Considering the circumstances surrounding the accident, there was no possibility of survival for the missing persons; however, their families and the surviving students could not accept the fact that they were dead until their bodies were actually found. One student stated: "I can never think that they are dead. I really do not have the feeling that the accident has actually happened." Another stated with conviction: "he was a good swimmer. Yes, he could easily swim 10 kilometers or so, so I think he got to the shore of some island."

Psychologically, such irrational feelings can be considered as denial. However, because the families of the missing persons also shared these feelings, a ritual or funeral, which would prompt a grief work, could not be held. For example, it was not until almost 1 year after the accident that the joint memorial service was held in January of the following year. Thus, for a long period of time, not only the victims but also the inhabitants in Uwajima were unable to accept the deaths of the missing persons. During this period the students continued to be tormented with the illusion that the missing people might be alive despite the clear fact that they would never come back. In other words, this can be referred to as a strong conflict between internal and external facts. As in cases of the Estonia ferry disaster reported by Taiminen and Tuominen (1996), the illogical psychic schema that one cannot accept a death until he or she sees the body may be unique to survivors of maritime accidents.

Unfamiliarity with Psychiatric Treatment

Generally speaking, prejudice against or a lack of understanding of psychiatric treatment or the mentally disabled has long existed in Japan. However, in recent years, the number of outpatients visiting clinics for psychiatric treatment has rapidly increased, and the prejudice or stigma against psychiatric illnesses has diminished considerably. Nevertheless, conservative areas still exist in large numbers. In such areas, a strict social norm teaches that being afflicted with a psychiatric illness is something very shameful. Uwajima City is a traditionally conservative area. Although the area enjoys a generous spiritual climate, prejudice against psychiatric illnesses is very deep-rooted. Moreover, the Uwajima Marine and Fisheries High School is essentially a school for seamen, and the students are required to be as strong "as men." In such a climate, it is very difficult for one to admit a psychiatric illness, not to mention to receive treatment for it.

Following our first examination, we provided the students with detailed information about PTSD or grief reactions and frequently urged them to receive some form of psychiatric treatment. Initially, many of them would not admit that they had a psychiatric illness, much less receive treatment. The period in which they could not attend school became prolonged, and ultimately many of the students began to visit local clinics and obtain psychiatric treatment.

6.2. Considerations on the Mental Status of Students at the Third and Fourth Examinations

Many of the students graduated from high school without a sufficient improvement in their condition. Although most strongly hoped to have a positive social life, in reality, most of them were suffering from various posttraumatic symptoms and remained socially withdrawn for several months after graduation. Following graduation, as mentioned above, the students' conditions gradually improved and they began to emerge from their isolation. Subsequently, at the 26-month examination, only one student showed symptoms of PTSD and depression.

We speculate that this remarkable improvement could have been the result of five factors. The first factor to be considered is that medication therapy (selective serotonin reuptake inhibitors, tricyclic antidepressants, or sleep inducers) was effective for many students. Although they initially refused to receive any psychiatric treatment, they established good relationships with the doctors through the repeated psychoeducational programs and eventually accepted treatment. Second, crisis intervention for suicide prevention, including immediate admission to a psychiatric hospital, was provided as soon as suicidal ideation was identified. Third, aggressive community care, such as daycare and family support programs, were carried out in order to promote the self-help function of the students and families. Fourth, we were able to establish strong relationships with many facilities, such as a healthcare center, schools, hospitals, and an employment agency. Finally, our encounters with the media were generally adequate and successful, and the media may have had a strong influence on the inhabitants' attitude toward the students.

In terms of caring for victims of a disaster, positive outcomes require comprehensive, well-integrated, and multidimensional intervention as well as individual treatment such as cognitive-behavioral therapy or medication. According to Raphael and Wilson (1993), "any proposed intervention should have well-defined goals, be targeted to those most in need, be flexible and adaptive to the realities of the disaster and postdisaster community, and reflect compassion, warmth, and empathy toward those who have been affected." The present intervention was developed according to these important principles.

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Structural and Functional Neuroimaging in Posttraumatic Stress Disorder

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1. Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric condition associated with a constellation of disabling behavioral and emotional symptoms, which occur in a proportion of individuals exposed to severe psychological trauma, such as combat, sexual abuse, natural disasters, and terrorism. For several years, we have pioneered research in neuroimaging of PTSD in Japan. Here we describe some of our findings, which include: (1) a structural magnetic resonance imaging (MRI) study in patients with PTSD recruited from the victims of the Tokyo subway sarin attack; (2) an event-related potential study and its association with structural MRI abnormality in the same sample; (3) a functional MRI study in an independent sample of Japanese PTSD patients.

2. Structural Abnormalities in PTSD

MRI studies using the manual tracing method have shown smaller than normal hippocampal volume in patients with PTSD. However, these studies have yielded inconsistent results, and brain structures other than the hippocampus have not been well investigated. A recently developed, fully automated method called voxel-based morphometry (VBM) enables an exploration of structural changes throughout the brain by applying statistical parametric mapping to high-resolution MRI. In the current study (Yamasue et al. 2003), we employed VBM to explore structural brain differences in the gray matter as well as the white matter between victims of the Tokyo subway sarin attack with and without PTSD. Based upon findings from previous neuroimaging studies, we hypothesized that patients with PTSD would demonstrate volume reduction in medial temporal regions (including the hippocampus) and medial prefrontal cortices (including anterior cingulate cortex, or ACC). The Tokyo subway sarin attack was caused by terrorists belonging to a cult named Aum

Shinrikyo in Japan on 20 March 1995. About 5500 victims were exposed to sarin, a poisonous gas, and 12 of the victims died. We recruited victims, with or without PTSD, who had never received psychiatric treatment for PTSD due to the attack. In addition, these subjects had little history of psychotropic treatment and no history of alcohol and substance abuse, which can be significant confounding factors in neuroimaging studies of PTSD.

Thirty-six victims were recruited from victims of the Tokyo subway sarin attack who were treated in the emergency room for acute sarin intoxication with follow-up at St. Luke's International Hospital in Tokyo, Japan. Out of the 36 victims, 9 were diagnosed as having PTSD related to the attack [1 man had current PTSD; 8 (4 men and 4 women) had past PTSD]. Ten more victims met more than one of the three symptom clusters of PTSD (including reexperiencing of the event, hyperarousal, and numbing), and 1 more was diagnosed as having current alcohol dependence; these 11 victims were excluded from further analysis. The remaining 16 victims (10 men, 6 women) did not have PTSD or any history of neuropsychiatric disorders. Thus, the final set of subjects in this study included 9 victims with (current or past) full PTSD and 16 victims without PTSD. Three of the 9 victims diagnosed as having PTSD had the following psychiatric comorbidity: current major depression ($n = 1$), current ($n = 1$) and past ($n = 1$) panic disorder with agoraphobia. Although 2 of the 9 victims diagnosed with PTSD had received benzodiazepines for insomnia or general anxiety over an approximate 3-month period 2-4 years earlier, the other 23 victims had never received psychiatric treatment before participating in this study. The MRI data were obtained using a 1.5-Tesla scanner (General Electric Signa Horizon Lx version 8.2, GE Medical Systems, Milwaukee, WI, USA). Voxel dimensions were $0.9375 \times 0.9375 \times 1.5$ mm. Image analysis was performed using ANALYZE PC 3.0 (Mayo Foundation, Rochester, MN, USA) and SPM 99 software (Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK).

An area with lower gray matter intensity in victims with PTSD compared with those without PTSD after controlling for the factors of age and sex was found within the left ACC [peak Montreal Neurological Institute (MNI) coordinates (mm) [x, y, z] = $[-8, 12, 32]$, $k = 113$, $z = 4.33$, corrected $P = 0.015$ with small volume correction (SVC) of 56.7 ml]. No other gray matter or white matter regions showed any significant intensity difference between the two groups. These results indicate diminished left ACC gray matter volume in victims with PTSD. It is unlikely that the volume reduction of ACC in patients with PTSD was solely the consequence of the neurotoxic effect of sarin on brain structure, because there was no significant difference in serum cholinesterase concentration between the two groups. (If anything, there was a trend toward slightly higher sarin exposure in the non-PTSD subjects).

Among the patients with PTSD, there was a significant negative correlation between total score on the Clinician-Administered PTSD Scale (CAPS) and left ACC gray matter intensity (peak coordinate [x, y, z] = $[-8, 12, 28]$, $z = 4.36$, corrected $P = 0.025$; SVC = 0.904 ml based upon $k = 113$ obtained in the main analysis) after controlling for the effects of age and sex. This result indicates that the reduced ACC gray matter volume showed a significant inverse relationship with PTSD severity.

Gray or white matter intensity was not significantly correlated with total CAPS score in any other brain regions.

In summary, morphological analysis using VBM revealed a significant regional volume reduction in the left ACC gray matter in traumatic survivors with PTSD compared with those without PTSD. This reduced ACC gray matter volume was significantly related to the severity of the disorder in PTSD subjects. No significant structural difference was found in any other region, including the medial temporal region. These findings are consistent with several other neuroimaging studies that have suggested dysfunction or neuronal loss in ACC in patients with PTSD (Bremner et al. 1999a; De Bellis et al. 2000; Shin et al. 2001). The results of the present and previous studies support a neuroanatomic model of PTSD that posits a failure of ACC to inhibit a hyperresponsive amygdala (Bremner et al. 1999b; Rauch et al. 2000; Shin et al. 2001). These considerations suggest that the ACC may be one of the most important regions in the pathophysiology of PTSD.

3. Relationship Between Structural and Electrophysiological Abnormalities

PTSD is associated with cognitive deficits, specifically impaired attention, such as difficulty in concentrating and sustaining attention on target tasks. These attentional problems in PTSD have been evaluated using auditory event-related potentials (ERPs). The P300 has been shown to reflect the ability to allocate attentional resources and update environmental context. The existing literature suggests that the P300 is reduced in patients with PTSD (McFarlane et al. 1993; Metzger et al. 1997), although these findings have not been uniformly replicated (Kimble et al. 2000). However, no study to date has evaluated neuroanatomical correlates of P300 abnormalities in PTSD, despite many independent publications of structural MRI abnormalities in PTSD such as those in ACC (Yamasue et al. 2003) and hippocampus (Gilbertson et al. 2002), brain regions thought to be associated with P300 generation. Consequently, we coevaluated auditory P3b and MRI in never-treated, comorbidity-free patients with PTSD following the Tokyo subway sarin attack (Araki et al. 2005). The victims of the same events who did not develop PTSD served as the control group. We performed correlational analyses between ERP indices and clinical symptoms, and structural MRI abnormalities of the ACC that we have recently reported in the current sample using VBM (Yamasue et al. 2003).

Out of the 47 victims recruited, 8 victims with PTSD and 13 victims without PTSD were identified. Six subjects with PTSD and 12 subjects without PTSD overlapped with the VBM study. The subjects performed an oddball task to elicit P300 in a soundproof room. They were presented with a series of auditory stimuli with a fixed interstimulus interval of 1500 msec. Eight-five percent of the stimuli were tones of 1000 Hz, and the other 15% were tones of 2000 Hz. The subjects were instructed to press a button upon hearing the infrequent, high pitch tones. Scalp elec-

troencephalogram was recorded at Fp1, Fp2, F3, Fz, F4, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, and Oz according to the international 10-20 electrode system, referenced to linked earlobes.

Regional voxel-based analyses of the images were performed in SPM99 after covarying for global normalization in an analysis of covariance (ANCOVA) model, which removes global gray matter intensity for each subject and normalizes the segmented brain images to the same total amount of gray matter while preserving regional differences in gray matter intensity. The P300 amplitudes at Pz were treated as the covariates of interest. To test hypotheses about regionally specific covariate effects, the estimates were compared using linear contrasts (positive or negative correlation).

Groups differed significantly in P300 amplitude (main effect of group: $F[1,19] = 4.80$, $P = 0.041$), while the group-by-electrode interaction was not significant ($F[4,76] = 1.21$, $P = 0.32$, epsilon = 0.70). For P300 latency, there was no significant main effect of group ($F[1,19] = 0.49$, $P = 0.49$) or group-by-electrode interaction ($F[4,76] = 0.35$, $P = 0.80$, epsilon = 0.80). In the PTSD group, there was a significant negative correlation between present score of the cluster C of the CAPS and P300 amplitude at Pz ($\rho = -0.850$, $n = 8$, uncorrected $P = 0.007$; corrected $P = 0.042$ after Bonferroni correction, two-tailed). Within victims with PTSD, there was a trend-level positive correlation between the amplitude of P300 at Pz and gray matter density in the left ACC (peak coordinate = $[-12, 32, 22]$, corrected $P = 0.077$, $z = 4.08$; SVC = 0.904 ml based upon $k = 113$ obtained in the main analysis of the VBM study). This result suggests that reduced ACC gray matter volume may underlie auditory selective attention deficits as indexed by P300 in patients with PTSD. In the other regions, gray or white matter density was not significantly correlated with the amplitude of P300. In contrast, there was no voxel within the left ACC correlated with the amplitude of P300 in victims without PTSD.

In summary, we replicated a significant reduction in P300 amplitude in the odd-ball task in PTSD patients who were victims of Tokyo subway sarin attack compared with those victims who did not develop PTSD. We found a significant correlation between P300 amplitude and avoidance/numbing cluster score of the CAPS. Furthermore, lower P300 amplitudes showed a trend toward significant correlation with lower gray matter voxel densities of ACC in victims with PTSD, whereas such an association was not present in victims without PTSD. Although P300 has multiple generators in humans, a smaller ACC volume in the individuals with PTSD may be proportional to lower functioning of this brain region, which is one of the generators of P300, thus resulting in a tighter structure-function relationship in the PTSD group. These results provide evidence that electrophysiological deficits in controlled attention in PTSD are linked to underlying brain morphological abnormalities.

4. Functional Abnormalities in PTSD

The purpose of this functional MRI study (Sakamoto et al. 2005) was to detect dysfunctional areas common to PTSD in the whole brain at high temporal and spatial resolution, and to obtain clues to elucidate the neural mechanisms of PTSD. In previous imaging studies of brain function in PTSD, narrations, photographs, or sounds of traumatic experiences have been directly presented to subjects, and brain activity during stimulation has been measured by positron emission tomography (PET) or single photon emission computed tomography (SPECT). However, because one of the major symptoms in PTSD patients is marked fear associated with traumatic experience, explicit presentation of traumatic stimuli may cause excessive distress and be difficult to perform in many subjects. The results of earlier studies have suggested functional abnormalities in the temporal area, occipital area, amygdala, cingulate gyrus, and frontal area during exposure to symptom provocation stimuli, but findings have been inconsistent among the studies.

The disagreement between the results of previous studies may be because excessively strong stimuli induced a variety of different reactions in subjects, or because changes in blood flow not directly associated with PTSD symptoms were also detected due to the long scan intervals used in the PET or SPECT measurements. Only a small number of studies avoided explicit presentation of traumatic stimuli. In these studies, masked images were presented for a very short period, which was below the conscious perceptual threshold, and measurements were performed by functional MRI with high temporal resolution. The regions of interest (ROI) that were examined in these studies were the amygdala and anterior cingulate gyrus or the occipital lobe. However, dysfunctions associated with PTSD are not limited to these areas, and studies that examine the whole brain are necessary. Most previous functional imaging studies of PTSD have used only combat veterans or victims of sexual abuse as PTSD subjects. This limitation of subjects is advantageous regarding subject homogeneity. However, PTSD results from various causes, such as accidents, terrorism and natural disasters. Hence, the investigation of functional abnormality common to PTSD arising from various causes is also important to elucidate the underlying neural mechanisms.

Sixteen patients with PTSD and 16 healthy persons participated in the following study. Traumatic images under the conscious perceptual threshold, including scenes of earthquakes, traffic accidents, ambulances, emergency rooms, and crimes, were presented to the participants. Although the images appeared as only flowering plants, traumatic images were inserted for a very short period. Each participant viewed the images for 4 min. This period comprised eight blocks (30 s each). The first, third, fifth, and seventh blocks were REST blocks, and the second, fourth, sixth, and eighth blocks were TRAUMA blocks. In each REST block, the images were changed in the following order: an image of a red cross below the conscious perceptual threshold (0.033 s), an image of flowering plants (0.167 s), and a black blank image (0.300 s). In each TRAUMA block, images were changed in the following order: an image of a traumatic scene below the cognition threshold (0.033

s), an image of a flowering plant (0.167 s), and a black blank image (0.300 s).

Functional images of both groups were evaluated for the whole brain. Separate analyses within the PTSD and healthy groups were performed using one-sample *t*-tests employing a random-effects model in SPM99. In addition, differences in brain activation between the PTSD and healthy groups were analyzed by a two-sample *t*-test. A level of $P < 0.01$ uncorrected for multiple comparisons with a minimum cluster size of 20 was chosen for the activation threshold. Correlations between activations and CAPS score or Dissociative Experience Scale-II (DES-II) score were evaluated in the PTSD group.

The areas activated by the masked traumatic stimuli were analyzed separately by one-sample *t*-tests in SPM99 for the PTSD and the healthy groups. In the healthy group, significant activations (uncorrected $P < 0.01$) associated with the masked traumatic stimuli was observed in the right inferior parietal lobule (Talairach coordinates: [44, -56, 48]; Brodmann Area [BA] 40; $z = 3.57$), left inferior parietal lobule ([-28, -76, 42]; BA 40; $z = 3.36$), right precentral gyrus ([28, -16, 52]; BA 4; $z = 3.55$), left precentral gyrus ([-28, -18, 54]; BA 4; $z = 3.33$), and left middle frontal gyrus ([-34, 20, 44]; BA 8; $z = 3.30$; [-16, 14, 26]; BA 9; $z = 3.19$). In the PTSD group, activation was not observed in these areas, but significant marked activation (uncorrected $P < 0.01$) associated with the masked traumatic stimuli was observed in the parahippocampal region, including left parahippocampal gyrus and tail of left hippocampus ([-14, -42, -4]; BA 19; $z = 3.79$). Differences in activation between the PTSD and healthy groups were analyzed by two-sample *t*-tests. The PTSD group showed significantly greater activation (uncorrected $P < 0.01$) in the left parahippocampal gyrus ([-12, 44, -4]; BA 19, $z = 3.15$), compared with the healthy group. The healthy group showed significantly greater activations in the left superior frontal gyrus ([-8, -2, 52]; BA 6; $z = 3.84$), left cerebellum ([-18, 72, -22]; $z = 3.81$), right cerebellum ([20, -46, -12]; $z = 3.67$), and right precentral gyrus ([28, -18, 56]; BA 4; $z = 3.10$), compared with the PTSD group. In the PTSD group, the percent fMRI signal change in the left parahippocampal gyrus/tail of left hippocampus (a region within a 10-mm radius from the peak of activation, [-14, -42, -4]) was significantly correlated with the dissociation symptom score on the DES-II (Pearson's $r = 0.499$, $P < 0.05$). These results imply that PTSD patients cannot inhibit hyperactivity in the limbic system associated with unconscious recollection of emotional memory induced by subliminally presented threatening stimuli. An important implication may be that our experimental design is potentially useful for clinical application, because we used both a physically and psychologically noninvasive method to detect differential activity in PTSD patients. Further sophistication of this paradigm would hopefully lead to objective diagnosis and monitoring of treatment response for PTSD.

5. Conclusions

Our studies using Japanese patients confirm that cortical abnormalities in PTSD are located in limbic regions and in medial prefrontal cortex, which regulates the limbic

system. Also, our VBM and fMRI results may be potentially useful for the clinical assay of PTSD. Future research should aim at a better convergence of knowledge from basic neuroscience and clinical studies, including: (1) further clarification of whether these abnormalities represent preexisting vulnerability factors or posttraumatic toxic changes or both; and (2) investigation of how genetic backgrounds, early environmental effects, or their interaction lead to the possible vulnerability.

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Functional Neuroimaging Research in Posttraumatic Stress Disorder

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1. Introduction

Neuroimaging research in posttraumatic stress disorder (PTSD) is only two decades old, but is rapidly expanding and evolving in methodological sophistication (Pitman et al. 2001; Hull 2002; Liberzon and Phan 2003). Earlier structural and symptom-provocation studies are giving way to hypothesis-driven cognitive activation studies, longitudinal and treatment studies, and translationally driven integrative studies. The focused review of functional neuroimaging research in PTSD that follows discusses findings to better understand the functional neuroanatomy underlying PTSD symptoms and pathophysiology. We begin with a selective review of the research on functional neuroanatomy of emotions that is specifically relevant to PTSD. The ensuing discussion links the two bodies of literature in order to provide a better understanding of the processing of threat-related emotions and how this informs our understanding of the brain mechanisms that subserve PTSD.

PTSD is characterized by exposure to life-threatening event/s associated with intense emotional reactions. Symptom clusters consist of *reexperiencing* the trauma (such as nightmares and intrusive memories), *avoidance and numbing* (avoiding trauma-related cues, feeling emotionally distant from loved ones), and *hyperarousal* (hypervigilance, sleep disturbances) (American Psychiatric Association 2000). Because these criteria are descriptive, atheoretical, and not confined to a specific diagnosis, it is possible that patients with different neurobiological subtypes will share the same diagnosis or that patients with similar neurobiological abnormalities may cross descriptive categorical boundaries. Neurobiological evidence does offer the potential to identify findings specific to trauma exposure and/or to PTSD's symptoms and pathophysiology, thereby helping to differentiate, define, and treat these disorders in a more meaningful way.

One powerful, noninvasive means of investigating brain function in PTSD is the use of relatively new neuroimaging methods such as magnetic resonance imaging (MRI) and positron emission tomography (PET). MRI methodology relies on spin properties of protons in human brain tissue to outline brain structures and estimate

their volume. Functional MRI (fMRI) uses the paramagnetic properties of oxy- and deoxyhemoglobin in conjunction with rapid acquisition sequences to create maps of blood-oxygen level dependent (BOLD) signal activity, i.e., blood flow reflective of neuronal firing, thereby allowing the study of neural processing of specific tasks. Single photon emission computed tomography (SPECT) and PET use radio-emitting isotopes of biologically relevant molecules to estimate blood flow, metabolic rate, receptor binding, and quantification and the assessment of pharmacological activity in vivo.

1.1. Functional Neuroanatomy of Emotions in Healthy Humans

An essential preamble to discussing neuroimaging studies of PTSD is to better understand the neural circuitry of emotional processing relevant to PTSD symptoms and pathophysiology. Although a number of different psychological processes (and underlying neural circuits) may be involved in the development and manifestation of PTSD, we choose to focus our discussion on three related areas of research that are particularly relevant to PTSD. These are: fear- and threat-related processing; cognitive-emotional interactions; and self and social aspects of emotion.

1.1.1. Fear-Related and Threat-Related Processing

The concept of a threat-related neural circuitry has emerged from animal studies of fear conditioning. It involves functionally connected brain regions such as medial prefrontal cortex (MPFC), rostral and subgenual anterior cingulate cortex (rACC and sgACC), insular cortex, amygdala, hippocampus, and thalamus. The respective roles of these structures are being further refined collectively by animal research and human brain lesion and neuroimaging studies (Adolphs et al. 1995; LeDoux 2000; Phan et al. 2002). There is also evidence for differential pathways underlying processing of threat-related stimuli rapidly but with less specificity (subcortical) versus processing that is relatively slower, more nuanced, and conscious (cortical) (LeDoux 2000). Functional neuroimaging studies have also attempted to further define this circuitry by probing implicated regions as well as exploring functional and effective connectivity between these regions.

One of the core symptoms of PTSD involves hyperarousal and hypervigilance to trauma-related as well as more generally aversive (trauma-unrelated) stimuli (Shalev et al. 1997; Liberzon et al. 1999a, 2000; Pitman et al. 1989, 1990a). There are striking similarities between conditioned fear responses in animals and some aspects of PTSD symptomatology (LeDoux 2000; Davis 1998; Lang et al. 2000). These observations have offered a starting point for exploration of PTSD-relevant neuroanatomy by identifying initial circuits of interest involved in fear conditioning. The past two decades have witnessed a growing body of parallel basic neuroscience and neuroimaging research addressing fear- and threat-related processing including perception, experience, conditioning, habituation, and extinction. We confine our discussion below to human neuroimaging studies.

Perception of Fearful Faces

A fearful facial expression is a signal to the observer that there is a potential threat. Recognition of fearful facial expressions likely involves neural circuits that are dedicated to threat perception. Thus, threat-related (fearful) facial expressions, along with facial displays of happiness, disgust, anger, and surprise, have been extensively utilized as stimuli in human neuroimaging studies of emotional circuitry. The amygdala and the extended amygdala have been identified as key structures of interest in light of their role in stimulus-reinforcer associations during fear conditioning, as observed in animal studies (LeDoux 2000; Davis 1998; Lang et al. 2000). Moreover, perception of emotionally valenced faces evokes amygdala activation, and this effect is seen robustly with fearful faces both when presented to subjects overtly (Breiter et al. 1996; Phillips et al. 1997; Morris et al. 1996, 1998a) as well as covertly by backward masking (Whalen et al. 1998). Thus, fearful (and other expressive) facial expressions are often used as “probes” of the amygdala region. These studies have repeatedly demonstrated the involvement of the amygdala region in the perception of emotionally valenced faces as well as involvement of the extended amygdala region in vigilance and salience detection (Davis and Whalen 2001).

Experience of Anxiety and Fear

A number of studies have focused on mood induction paradigms to study the neural correlates of the emotional experience itself. Such studies have employed pictures of varying affective significance, video clips, self-generated scripts, and pharmacological agents such as procaine. They have involved induced sadness (Pardo et al. 1993; George et al. 1995; Lane et al. 1997; Liotti 2000), anger (Dougherty et al. 1999), guilt (Shin et al. 2000), and anxiety (Benkelfat et al. 1995; Ketter et al. 1996; Paradiso et al. 1997; Servan-Schreiber et al. 1998; Liotti 2000; Kimbrell et al. 1999). The most consistent finding across these studies involves the activation of overlapping anterior paralimbic structures (posterior medial orbitofrontal, anterior temporal, anterior cingulate, and insular cortex) and the extended amygdala during the experience of anxiety and of other negative emotions, suggesting shared pathways in the experience of different negative emotions.

Fear Conditioning and Aversive Emotional Memory

Human brain lesion studies have implicated the amygdala in fear conditioning (Bechara et al. 1995; LaBar et al. 1995) as well as in the recognition of fear expression (Adolphs et al. 1994, 1999). Animal research has implicated the amygdaloid complex in modulating the formation of memory for aversive learning (McGaugh 1996), and functional neuroimaging studies in humans also support this notion. In an ^{18}F -fluoro-2-deoxyglucose (FDG) PET study of eight right-handed male subjects, Cahill et al. (1996) demonstrated that the regional metabolic rate of the right amygdaloid complex was highly correlated with the number of emotionally arousing film clips recalled, whereas there was no such correlation to the emotionally neutral film clips.

The fear-conditioning phenomenon has been specifically addressed in several human neuroimaging studies (Buchel and Dolan 2000). In an innovative classical conditioning study using overt and masked faces combined with [^{15}O]- H_2O PET in ten right-handed healthy male subjects, Morris et al. (1998a) demonstrated that the amygdala was activated during CS+ trials [conditioned stimulus (CS) followed by aversive unconditioned stimulus (US)], but not during CS- trials (CS not followed by US). The same investigators also found that lateralization of the amygdala signal was dependent on automatic or subconscious (masked CS+ minus masked CS- : right amygdala) versus conscious (unmasked CS+ minus unmasked CS- : left amygdala) presentation of the CS (Morris et al. 1998a). Using an auditory CS, the same group demonstrated correlations between activations in the learning-related areas of the auditory cortex and other structures such as bilateral amygdala, anterior cingulate gyrus, and bilateral insula (Morris et al. 1998b). Two fMRI studies, conducted by separate groups, using aversive delay conditioning paradigms, showed greater activation of the ACC and amygdala to be associated with the CS+, and a CS+-related signal decrement over time (LaBar et al. 1998; Buchel et al. 1998). A later study used a trace conditioning paradigm and demonstrated anterior hippocampal activation in addition to amygdala and ACC, with similar temporal decrement, which was interpreted as reflecting the role of the hippocampus in maintaining a representation (memory) of the association (Buchel et al. 1999). Finally, a recent study employing event-related fMRI (er-fMRI) compared the delay and trace conditioning methods. Conditions were CS+, CS-, and CS₁₀ (US and CS separated by a 10-s trace interval); pain ratings, skin conductance response (SCR), and shock expectancy were recorded. The results showed overlapping patterns of activation in the ACC, medial thalamus, and visual cortex during delay and trace procedures, with additional recruitment of the hippocampus during trace conditioning interpreted by the authors as evidence for a hippocampal role in this phenomenon (Knight et al. 2004). Thus, it appears that the human fear-conditioned state, which is relevant to aspects of the symptomatology of anxiety disorders including PTSD, involves the ACC, amygdala, and hippocampus.

Habituation and Extinction

Temporal decrement in activation in brain regions involved in threat processing can occur due to a number of mechanisms, including habituation and extinction, and this has been a focus of attention for both basic science and in vivo neuroimaging studies. Habituation refers to a process where there is rapid attenuation of a neural response simply as a function of repeated presentation of the stimulus, whereas extinction refers to the decrement in signal due to novel association learning, e.g., CS no longer signifies US. Both habituation and extinction are adaptive, because they allow reallocation of resources from stimuli that have stopped signifying threat to stimuli that signal novel threat and are salient to the survival of the organism.

In two earlier neuroimaging experiments that investigated the human brain response to emotionally expressive facial stimuli presented overtly or covertly (fearful or happy faces masked with a neutral face such that subjects consciously perceive only the neutral face), the authors found that the amygdala response to emo-

tionally valenced faces rapidly habituates regardless of mode of presentation (Breiter et al. 1996; Whalen et al. 1998). Another group reported that only two repetitions of complex visual stimuli resulted in reduction in regional cerebral blood flow (rCBF) bilaterally in the secondary visual cortex and in the right medial temporal cortex, including the amygdala and the hippocampus (Fischer et al. 2000). On the other hand, repeated stimuli presentations resulted in increased rCBF in the thalamus. The same group used repeated presentations of fearful and neutral faces and replicated signal decrement in the right amygdala and hippocampus, as well as bilateral medial/inferior temporal cortex (Fischer et al. 2003). However, this was not specific to the expression condition (fearful vs. neutral) (Gottfried and Dolan 2004). Similarly Wright et al. (2001) used repeated presentations of emotional facial expressions and reported significant fMRI signal decrement in the left dorsolateral prefrontal (DLPFC) and premotor cortex, and the right amygdala. The DLPFC interestingly showed greater signal decrement in the happy versus fearful condition. The authors interpreted these findings as reflecting possible differential responsiveness of prefrontal versus subcortical structures. Our laboratory has also investigated the question of habituation in prefrontal cortical regions specifically and demonstrated rostral ACC habituation with repeated emotional picture (aversive minus neutral/blank) presentation (Phan et al. 2003).

Finally, a recent trial-related fMRI study investigated the process of extinction that has been suggested to be dependent on intact function of prefrontal cortex in animal studies (Morgan et al. 1993; Morgan and LeDoux 1995). The authors used an olfactory aversive conditioning and extinction paradigm and demonstrated that discrete regions of the rostral and caudal OFC and lateral amygdala are preferentially activated during the extinction process (Gottfried and Dolan 2004). They also demonstrated through an innovative design that the CS+ retains access to representations of the US in distinct regions of the ventral PFC even as the extinction learning process proceeds (supporting formation of a new association rather than erasing of the old one), which is consistent with evidence from animal studies.

Thus, the evidence from human neuroimaging studies discussed above implicates subregions of the medial prefrontal cortex (MPFC) and orbitofrontal cortex (OFC), subdivisions of the ACC, the extended amygdala, the hippocampus, and nuclei of the thalamus in the processes of fear perception and conditioning, aversive learning, habituation, and extinction. Identification of these key components in threat processing circuitry has important implications for both the understanding of anxiety and fear in humans as well as future research in anxiety disorders such as PTSD.

1.1.2. Cognitive-Emotional Interactions

The concept of cognitive-emotional interactions is receiving increased attention in neuroimaging research due to the importance of these processes in the regulation of affective states and in decision-making processes, and the obvious implication of both for anxiety disorders and PTSD.

Cognitive Appraisal of Emotions

There is increasing evidence that cognitive components of tasks modulate both subjective emotional responses and activity in emotion processing areas. Studies using emotional stimuli have often involved either passive viewing or stimulus rating to access characteristics such as type of emotion, intensity, etc. There is accumulating evidence that even the simple process of labeling or rating an emotional state (appraisal) can effectively modulate the strength of the experienced emotion. This is particularly interesting in the view of the fact that PTSD subjects have difficulties identifying and describing their emotions (a phenomenon labeled alexithymia).

In one of the earlier neuroimaging studies to address this phenomenon, Hariri et al. (2000) studied healthy subjects as they matched expressive faces or identified the affect (linguistic labeling). Matching angry or frightened expressions was associated with increased rCBF in the left and right amygdala. Labeling these same expressions was associated with a diminished rCBF response in the amygdala that correlated with a simultaneous increase in rCBF in the right prefrontal cortex (Hariri et al. 2000). Our laboratory examined the effect of adding a cognitive task on emotionally responsive regions by exposing ten healthy female volunteers to aversive or neutral emotional pictures while measuring brain activity with [^{15}O]- H_2O PET and peripheral psychophysiology (Liberzon et al. 2000). The cognitive task manipulation was achieved by having the subjects rate the pictures or perform a recognition memory task. Aversive (relative to neutral) pictures increased cerebral activity in bilateral amygdala, thalamic/hypothalamic area, midbrain, and left lateral prefrontal cortex, along with greater skin conductance responses (SCR). Limbic activation was significantly lower during the recognition condition compared with simple appraisal, suggesting that increased cognitive demand further modifies both subjective emotional responses and level of limbic activity. In a follow-up experiment, we studied rCBF response in healthy subjects comparing an appraisal to a passive viewing condition (Taylor et al. 2003). Subjects were presented subjects with aversive and neutral pictures from the International Affective Picture System (IAPS) (Lang et al. 1997), while they performed two tasks: one of passive viewing (PSVW) and another of appraisal ("rating"-RTNG). RTNG resulted in reduction in intensity of sadness and less activation of the right insula/amygdala and left insula compared with PSVW. On the other hand, RTNG, but not PSVW, was associated with increased activation of the dorsal MPFC and the ACC. These findings demonstrated the involvement of the DMPFC and ACC in cognitive tasks (appraisal) and suggest modulating effects on emotion-related structures such as the amygdala and insula. This is consistent with findings from animal research showing a top-down influence of the medial PFC over the amygdala in fear conditioning, an experimental paradigm relevant to emotion modulation (Morgan and LeDoux 1995). In concert, Hariri et al. (2003) replicated their earlier finding, demonstrating that perceptual processing of IAPS stimuli was associated with a bilateral amygdala response, whereas cognitive evaluation of these same stimuli was associated with attenuation of this amygdala response and a correlated increase in response of the right PFC and the ACC.

Cognitive Modulation of Emotions - Reappraisal

A number of studies have directly examined the effect of voluntary cognitive manipulation of experienced emotion (reappraisal). These studies are relevant to the study of PTSD, in which emotion regulatory processes are presumed to be dysfunctional. Using an event-related fMRI paradigm, Ochsner et al. (2002) studied healthy female subjects by means of aversive IAPS pictures. Subjects were instructed to “attend” (be aware of feelings elicited by stimuli) or to “reappraise” (reinterpret stimuli so that it no longer elicited a negative emotional response). Reappraisal of highly negative scenes was successful, and the contrast of reappraise > attend showed increased activation of dorsal and ventral left lateral prefrontal cortex (DLPFC and VLPFC), dorsal medial PFC (DMPFC), left temporal pole, right supramarginal gyrus (SMG), and left lateral occipital cortex. The attend > reappraise contrast showed increased activity in the right amygdala, left posterior insula, left medial orbitofrontal cortex (MOFC), right medial occipital cortex, and right inferior parietal cortex. Greater activation in the right ACC and SMG correlated with greater decreases in negative affect (greater reappraisal success); left ventral PFC activation during reappraisal was inversely correlated with activity in the amygdala. Thus, effective reappraisal resulted in increased activation in LPFC and MPFC, regions implicated in working memory, cognitive control, and self-monitoring, and in decreased activation of M-OFC and amygdala, regions implicated in emotion processing.

In a follow-up fMRI study in healthy female volunteers, Ochsner et al. (2004) demonstrated that both up (voluntarily increase) and down (voluntarily decrease) regulation of negative emotion are possible, and that this recruits prefrontal and ACC regions implicated in cognitive control. Amygdala activation was modulated up or down depending on the regulatory goal. Up regulation uniquely recruited right lateral and orbital PFC. Self (internal)-focused regulation recruited medial PFC regions whereas situation (external) -focus regulation recruited lateral PFC regions (Ochsner et al. 2004). In a recent fMRI study, Phan et al. (2005) showed highly aversive and arousing pictures from the IAPS to healthy subjects, who were instructed to either “maintain” (feel naturally) or “suppress” (by positive reframing or rationalizing) negative affect. Successful reduction of negative affect was associated with increasing activation of dorsomedial PFC [Brodmann area (BA) 8], dorsal ACC (BA32), dorsolateral PFC (BA9), lateral OFC (BA11), and ventrolateral PFC/inferior frontal gyrus (BA46/44), and with decreasing activity in the left nucleus accumbens, left lateral PFC (BA46) and left extended amygdala. Additionally, right dorsal ACC (BA32), right anterior insula, bilateral dorsolateral PFC (BA8), and bilateral ventrolateral PFC (BA46/10) activity inversely correlated with the intensity of negative affect.

1.1.3. Self-Relatedness and Social Emotional Processing

Social interactions form a key component of human functioning, and the success of these interactions often determines long-term survival and reproductive success. It is logical, therefore, that this important function be subserved by dedicated neural

resources for the processing of social stimuli. Indeed primate and human lesions studies have implicated the MPFC, OFC, amygdala, and other regions in processing social and related stimuli. From a psychiatric perspective, the ability to reflect upon one's own emotional state as well as to process social stimuli are of interest, given that the manifestations of PTSD include difficulty regulating one's emotions, difficulties with functioning interpersonally and in social situations, and that interpersonal trauma results in the highest rates of PTSD (Kessler et al. 1995). For these reasons, the reflection upon and attribution of the "self" versus the "other," as well as other aspects of socially relevant emotional processing, is receiving increasing attention from the both neuroscience and the psychiatric community. For the purposes of this discussion, social emotional processing includes perception and recognition of social cues, social judgment and attribution, regulation of perception, and experience via appraisal and social interactions (Ochsner 2004).

A number of neuroimaging studies in recent years that have focused on the circuits that process self versus other attributions, as well as social stimuli, have highlighted the roles of MPFC, superior temporal sulcus, and OFC (Castelli et al. 2000; Gusnard et al. 2001). We briefly discuss here findings from two relevant studies that we conducted in this area. In the first study, we used aversive, positive, and neutral IAPS pictures in a trial-related fMRI design to compare the neural substrates underlying the assessment of the emotional intensity of the pictures versus the self-relatedness of their content, in healthy, right-handed volunteers (Phan et al. 2004a). Individualized subjective ratings over these two dimensions (obtained postscan) were correlated with brain activity in a parametric factorial analysis. The appraisal of self-relatedness specifically engaged the medial PFC and recruited the DMPFC and insula as the extent of self-relatedness increased. On the other hand, the amygdala activation was specific to affective judgment of emotional intensity. Both increasing emotional intensity and self-relatedness activated the nucleus accumbens. These findings suggest that appraisal of self-relatedness specifically recruits the medial PFC, a region that is implicated in the symptomatology and possibly pathophysiology of PTSD, as discussed below in the section on neuroimaging studies in PTSD.

Finally, in a recent study, we extended our investigation of the neural substrates of emotion to the processing of social versus nonsocial stimuli in 12 healthy, right-handed volunteers (Britton et al. submitted). In a novel paradigm, subjects viewed short video segments that evoked emotions that were categorized as "social" (humor, sadness) or "nonsocial" (appetite, physical disgust). Following the video, static frames extracted from the video were viewed for 30 s to help subjects maintain the emotions evoked by the video clip; during this period fMRI images were acquired. Nonsocial and social emotional experiences resulted in partially overlapping but somewhat separate neural patterns. All conditions activated the thalamus. Social positive and social negative conditions activated amygdala/sublenticular extended amygdala, superior temporal gyrus, hippocampus, and posterior cingulate, whereas nonsocial positive and nonsocial negative conditions activated insula and visual cortex. Additional activations depended on both social context and valence: amygdala (nonsocial negative); ACC (nonsocial positive and social negative); OFC and

nucleus accumbens (social positive). These findings highlight the importance of sociality in human emotions.

1.2. Functional Neuroanatomy of PTSD

Over the past two decades, neuroimaging studies of PTSD have explored structural abnormalities via computed tomography and MRI-based morphometry, neurochemical changes via magnetic resonance spectroscopy (of ACC and hippocampi), and functional alterations via fMRI, PET, and SPECT symptom provocation studies. These studies have compared PTSD subjects with trauma-exposed and/or non-trauma-exposed control subjects without PTSD. In the review that follows, we restrict our discussion to findings from functional neuroimaging studies of blood flow, BOLD effects, and receptor function. More recently, the original symptom provocation design has been further refined by paradigms that account for individual variance based on psychophysiological responsivity (Lanius et al. 2002). Newer design strategies have included connectivity analyses, correlation with symptom severity, neurocognitive activation, and receptor functioning. The focused discussion of the findings below is organized thematically under the following categories: (1) resting state studies; (2) symptom capture studies; (3) correlation between regional brain activity and severity of illness, and functional connectivity analyses; (4) cognitive neuroscience studies; and (5) receptor studies.

1.2.1. Resting State Studies

Resting state differences in rCBF are seldom reported in the psychiatric literature in general and the PTSD literature in particular. One possible limiting factor is that investigation of these states is not readily amenable to fMRI investigation, but requires SPECT or PET, although proxies of resting states can be investigated with fMRI. Another explanation of the paucity of resting state data is that it is difficult to demonstrate significant differences in resting state in these conditions, and negative findings are rarely published (publication bias). In a resting state study in PTSD, Bonne et al. (2003) compared subjects with PTSD ($n = 11$) with trauma-exposed subjects without PTSD ($n = 17$) and nontraumatized healthy controls ($n = 11$) using hexamethylpropyleneamineoxime (HMPAO) SPECT 6 months after the trauma. They found increased regional blood flow in the cerebellum in the PTSD group compared with both control groups. They also found higher rCBF in right precentral, superior temporal, and fusiform gyri in PTSD compared with healthy controls. Furthermore, cerebellar and extrastriate rCBF positively correlated with measures of depression and PTSD severity. Cortisol level in PTSD was negatively correlated with medial temporal lobe perfusion. Anterior cingulate perfusion and cortisol level were positively correlated in PTSD and negatively correlated in trauma survivors without PTSD.

1.2.2. Symptom Capture Studies

Symptom capture studies of patients with PTSD have employed trauma-related stimuli of an autobiographical nature (e.g., narrative scripts of their personal trauma) or general nature (e.g., combat-related pictures and sounds that are not personalized to their experience). Both types of stimuli reliably induce psychophysiological changes, such as elevated heart rate, skin conductance, and plasma catecholamines, in PTSD compared with various non-PTSD control subjects (Pitman et al. 1989, 1990a; Pitman and Orr 1990; Shalev et al. 1997; Liberzon et al. 2000). This finding suggests that the development of PTSD symptoms following trauma exposure is associated with neurobiological changes in stress/alarm systems including the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system. Clearly, these changes are coordinated by central neural mechanisms. Symptom capture studies provoke symptoms while attempting to capture the underlying neural substrates. As such these are the most abundant studies in PTSD and involve several modalities including SPECT, PET, and fMRI.

Rauch et al. (1996) first used individualized trauma scripts and [^{15}O]- H_2O PET to provoke symptoms in a small and heterogeneous group of eight PTSD subjects. In the provoked versus control (neutral and teeth clenching) contrast increased rCBF was observed in the right medial (posterior) OFC, insular, anterior temporal polar and medial temporal cortex, as well as in the secondary visual cortex (provoked vs. neutral) and anterior cingulate and amygdala (provoked vs. teeth clenching). These findings were interpreted as implicating limbic and paralimbic involvement in PTSD and sensory cortical involvement in visual imagery (reexperiencing) phenomena. Another study by the same group used combat-related, emotionally negative, and neutral pictures paired with verbal descriptions (imagery) to study the specificity of emotional processing in combat veterans with and without PTSD ($n = 7$ each group), as well as to differentiate visual perception from visual imagery (Shin et al. 1997). The ACC and right amygdaloid regions were activated in the combat imagery versus combat picture viewing contrast, but the ACC was deactivated in the combat image viewing versus neutral image viewing in PTSD subjects. Healthy controls also showed a relative deactivation in the combat perception versus the negative perception contrast.

An important limitation of these early study designs was the absence of appropriate control groups, which limited their ability to differentiate changes that occurred as a result of the trauma exposure alone from changes relating to the development of PTSD. Our laboratory studied three groups to address this question, including combat PTSD subjects ($n = 14$), combat-exposed subjects without PTSD ($n = 11$), and combat-unexposed healthy subjects (Liberzon et al. 1999a). Subjects were exposed to trauma-related stimuli (combat sounds) or white noise in two counterbalanced sessions. Only PTSD subjects showed amygdala activation in the main contrast (combat sounds–white noise); all three groups showed a common rACC/MPFC activation site. Bremner et al. (1999a) measured rCBF in 20 combat veterans (10 with and 10 without PTSD) using combat-related pictures and sounds. Exposure to combat-related material resulted in increased activation in ACC in healthy

controls (compared with PTSD subjects) and deactivation in subcallosal/subgenual (sc/sg) ACC and MPFC in PTSD subjects. In another symptom provocation PET study of women with histories of childhood sexual abuse (CSA) (10 with and 12 without PTSD) performed by the same group, the PTSD group showed rCBF increases in superior and middle frontal gyri, posterior cingulate cortex (PCC), and motor cortex, and deactivations in scACC, visual association cortex, and right hippocampus, with failure to activate rACC (Bremner et al. 1999b). Prefrontal rCBF abnormalities were interpreted to be correlates of resistance to extinction in PTSD subjects. Another group studied 16 subjects with CSA, half of whom had PTSD, with a script-driven imagery paradigm and PET (Shin et al. 1999). The traumatic versus neutral imagery contrast revealed greater increases in rCBF in the OFC and temporal poles and deactivation of the medial prefrontal and left inferior frontal (Brocas) areas in the PTSD group versus the non-PTSD group.

Two fMRI studies using different paradigms (symptom provocation vs. cognitive activation) also demonstrated reduced activation of the MPFC (BA 10, 11) and ACC (BA 32) in PTSD subjects compared with traumatized controls (Lanius et al. 2001; Shin et al. 2001). This has been interpreted as evidence of dysfunctionality in higher cortical structures important in the regulation of emotional responses. These areas have rich connections with the amygdala region, which is involved in threat-related processing. Extending this to an understanding of PTSD symptomatology, the failure of these higher regulatory structures to modulate the activity of the amygdala and related subcortical structures is thought to be related to the exaggerated emotional responses seen in PTSD. Indeed there is some evidence from human neuroimaging studies to support the extension of animal research findings of functional connectivity between medial prefrontal cortical structures and the amygdala (Morgan and LeDoux 1995).

Cortical abnormalities were also studied by Hendler et al. (2001), who reported findings from an fMRI study of combat veterans with and without PTSD. Subjects viewed pictures with and without combat content in repeated versus novel presentations. Repeated presentations of the same combat visual stimuli resulted in less BOLD signal decrease in the lateral occipital cortex in PTSD subjects (vs. non-PTSD), suggestive of impaired habituation of the response to trauma-related stimuli.

These reports of decreased, insufficient, or altered cortical activity raise the interesting question of how cortical and subcortical findings are related. Pertinent to the issue of causality, are top-down or bottom-up processes involved? Authors of PET symptom provocation study proposed a bottom-up mechanism based on increased rCBF response in primary sensory cortex, right amygdala, and periaqueductal grey area (PAG) adjacent to the pons, although the lack of appropriate controls in this study limits specificity of this finding and interpretation to PTSD (Pisioti et al. 2002). Another script-driven symptom provocation fMRI study compared the responses of ten traumatized subjects with PTSD (sexual abuse/assault and MVA) with ten traumatized subjects without PTSD. The PTSD subjects showed significantly less activation of the ACC (BA 32), medial frontal cortex, and thalamus in both the traumatic as well as nontraumatic emotional states (sad, anxious)

(Lanius et al. 2003).

More recently, Shin et al. (2004a) studied rCBF in Vietnam veterans with PTSD ($n = 17$) and without PTSD ($n = 19$) using script-driven imagery (personal traumatic and neutral) and PET. In the traumatic versus neutral comparison, the PTSD group showed rCBF decreases in the medial frontal gyrus, which were present in both the male combat veteran (MCV = 7) and female nurse veteran (FNV = 10) subgroups and were inversely correlated with rCBF changes in the left amygdala and the right amygdala/periamygdaloid cortex. Only the MCVs showed increased rCBF in left amygdala. Furthermore, in the traumatic condition, for both subgroups with PTSD, symptom severity (CAPS score) was positively related to rCBF in the right amygdala and negatively related to rCBF in medial frontal gyrus after controlling for depression severity scores.

Our laboratory recently conducted a [^{15}O]- H_2O PET, script-driven imagery study of emotionally evocative and neutral autobiographic events in 16 combat veterans with PTSD (PP); 15 combat veterans without PTSD (CC); and 14 healthy, aged-matched, noncombat control subjects (NC). This paradigm allowed us to investigate both changes that are trauma related (PP vs. NC and CC vs. NC) and PTSD specific (PTSD vs. CC). All subjects deactivated the MFC and activated the insula (traumatic/stressful > neutral scripts). PTSD patients deactivated the rACC more than both control groups (CC and NC) who additionally showed ventromedial PFC (VMPFC) deactivation. Trauma exposure (i.e., PP and CC groups) was associated with decreased amygdala activity compared with noncombat controls (NC). These findings suggest that patterns observed only in the PTSD group may reflect neural substrates specific to PTSD whereas trauma-specific patterns may represent potential compensatory changes to traumatic reminders (Britton et al. 2005).

1.2.3. Correlation Between Regional Brain Activity and (Cross-Sectional) Symptom Severity; Functional Connectivity Analyses (FCAs)

One strategy that has been used to understand the phenomenology of symptom genesis in PTSD is the correlation of imaging findings with measures of symptom severity. Osuch et al. (2001) correlated rCBF response with flashback intensity in a personalized, script-driven imagery PET paradigm in eight chronic PTSD subjects. rCBF correlated directly with flashback intensity in the brainstem, insula, and hippocampus and inversely in the prefrontal, right fusiform, and medial temporal cortices. Similarly Lanius et al. (2002) reported that seven CSA subjects with PTSD and concomitant dissociative responses to symptom provocation by scripts had increased activation in the ACC, MPFC, and several other cortical areas. None of these activations correlated with either dissociative or flashback intensity. The presence of significant comorbidity in a very small sample limits meaningful interpretation of these findings.

It is increasingly evident that different brain regions act in concert during the performance of cognitive-emotional tasks. This understanding can be extended to complex neuropsychiatric disorders involving symptoms that reflect abnormalities in cognitive-emotional processes. Recently investigators have been applying ad-

vanced statistical methods to identify interregional brain activity correlations (termed functional connectivity), or the influence of one brain region over another (effective connectivity), in the context of a specific task (Friston et al. 1993, 1996). While these methods may provide useful information about neural circuitry, they are still under development and should be considered preliminary with regard to making neurobiological interpretations. A few recent studies have applied these methods to neuroimaging studies of PTSD. Gilboa et al. (2004) studied 20 individuals with a history of civilian trauma, 10 of whom were diagnosed with PTSD, using symptom provocation (autobiographical trauma-related and neutral scripts) and [^{15}O]- H_2O PET. The patient and control groups were relatively well matched, except 3 patients were on clonazepam at the time of the study. A multivariate analysis technique (partial least squares) was used to identify brain regions whose activity covaried with two reference (“seed”) voxels, one in right PFC (BA10), the other in right amygdala (both derived from a preliminary task). The authors reported a significant influence of the amygdala (activity) on the visual cortex, subcallosal gyrus, and anterior cingulate in the PTSD subjects but not in the trauma-exposed controls (effective connectivity). They were, however, unable to find support for the failure of inhibition of the ACC over the amygdala (the FCA with the right amygdala seed revealed a positive salience of the ACC in both groups instead of the expected negative salience in the PTSD group).

Lanius et al. (2004) also used FCAs on data gathered during fMRI script-driven symptom provocation experiments in 11 subjects with PTSD due to sexual abuse/assault or MVA, and 13 trauma-exposed subjects without PTSD. Comparison of connectivity maps at a right ACC coordinate showed greater correlations in PTSD subjects (vs. controls) in the right PCC (BA29), right caudate, right parietal lobe (BA7 and 40), and right occipital lobe (BA19). Subjects without PTSD had greater correlations (vs. PTSD subjects) in the left superior frontal gyrus (BA 9), left anterior ACC (BA32), left striatum (caudate), left parietal lobe (BA40 and 43), and left insula (BA13). The differently lateralized findings between the groups were interpreted by the authors to possibly account for the differential access to verbal memories of the trauma observed in PTSD patients.

1.2.4. Cognitive Neuroscience Studies

One general limitation of symptom provocation paradigms is that they are confounded by the invocation of both specific symptoms and nonspecific anxiety responses. Cognitive activation studies refer to studies that probe implicated brain structures using a neurocognitive task that is expected to activate that region (a “probe”). In clinical neuroscience such probes can be used to compare the responses of implicated regions in patient and control groups. The ability to selectively do this without eliciting symptoms provides a specific advantage. In the earliest of such studies, Semple et al. (1996) used [^{15}O]- H_2O PET and challenge with an auditory attentional task in eight PTSD patients and eight healthy controls with substance abuse and reported 8%–11% lower rCBF in the right angular gyrus during the attentional task. Several methodological issues limited interpretation of this finding,

including small sample size, substance abuse comorbidity, lack of trauma-exposed control subjects, and the potentially confounding contribution of baseline rCBF differences. An innovative study compared amygdala responses in nine PTSD subjects with those of eight combat-exposed, nonPTSD subjects using a previously validated masked faces paradigm (Rauch et al. 2000). Fearful versus happy masked faces contrasts revealed exaggerated amygdala responses in the PTSD subjects. Moreover, the magnitude of these responses distinguished PTSD subjects with 75% sensitivity and 100% specificity. These findings are significant in that they provide evidence for increased amygdala responsivity to threat-related (but not necessarily trauma-related) stimuli in PTSD, as well as the ability of nonconscious threat-related stimuli to elicit such a response in PTSD.

Another group investigated mechanisms of updating working memory in PTSD using [^{15}O]- H_2O PET and a variant of the n-back task (detection of trauma-unrelated target words under fixed and variable conditions; only the variable condition required target updating) in ten patients with PTSD (mostly civilian trauma) compared with ten healthy subjects (Shaw et al. 2002). The result of the analysis examining functional connectivity during the working memory task revealed increased activation in bilateral (B/L) inferior parietal lobules and left precentral gyrus, and reduced activation in inferior medial frontal lobe, B/L middle frontal gyri, and right inferior temporal gyrus in the PTSD group relative to the control subjects. This was interpreted by the authors to represent a compensatory increase in dependence on nonverbal memory networks in PTSD patients, due to impairments in verbal working memory updating. Bremner et al. (2004) used the Stroop task (color stroop, emotional stroop, and control task) and [^{15}O]- H_2O PET to probe ACC function in women with early CSA-related PTSD ($n = 12$) and abused women without PTSD ($n = 9$). The PTSD group had a relative decrease in ACC blood flow during the emotional but not the color stroop task. The color stroop task elicited increased rCBF in the ACC (BA 24 and 32) in both groups. In non-PTSD subjects (vs. PTSD) the color stroop task was also associated with greater increases in rCBF in right visual association cortex (BA 19), cuneus, and right inferior parietal lobule (BA 40), as well as greater decreases in right superior temporal gyrus (BA 38) and orbitofrontal cortex (BA 11). The authors interpreted these findings as supporting the specific involvement of the ACC in PTSD with regard to the processing of trauma-relevant stimuli.

Shin et al. (2004b) used a word-stem completion task and PET to study rCBF in the hippocampus in 16 firefighters (8 with PTSD). Subjects completed a three-letter word stem with words encoded during a preceding training session. There were two classes of words: deeply encoded/high recall and shallow encoded/low recall. The PTSD group demonstrated greater rCBF in the hippocampi (bilateral) across conditions. In the main contrast of interest (high vs. low recall) the PTSD group (vs. control group) showed significantly smaller rCBF increases in the left hippocampus, which was primarily driven by relatively elevated rCBF in the low recall condition. PTSD subjects also had smaller right (and a trend toward smaller left) hippocampal volumes. In another study, the same group used emotional facial expressions and fMRI to compare BOLD responses in 13 men with PTSD and 13 trauma-exposed men without PTSD (Shin et al. 2005). The PTSD group showed increased

amygdala responses and decreased medial prefrontal cortex responses to overt fearful (vs. happy) facial expressions. BOLD signal changes in the amygdala in the PTSD subjects were negatively correlated with signal changes in the medial prefrontal cortex. Additionally, BOLD signal changes in the medial prefrontal cortex were inversely correlated with symptom severity (CAPS). In a recent block design fMRI study, investigators examined the time course of amygdala responses to trauma-relevant negative, panic-relevant negative (negative control condition), positive/safety, and neutral words in 9 predominantly sexual assault PTSD patients as compared with 14 healthy controls (Protopopescu et al. 2005). The PTSD group (compared with healthy controls) showed an increased left amygdala response to trauma-relevant negative versus neutral stimuli in the first two (but not last two runs), and this response correlated with the symptom severity (CAPS total score). Healthy controls showed the opposite pattern. Similarly opposite patterns were found between PTSD subjects and healthy controls to panic words, i.e., PTSD subjects demonstrated increased left amygdala BOLD response to panic words (controlled for neutral words) late, but not early, in the study, whereas healthy controls showed the opposite response. The authors interpret the former finding as abnormal sensitization in PTSD. However, when viewed as a habituation phenomenon, the findings are counterintuitive, in that the PTSD group showed habituation to trauma-relevant words (controlled for neutral words) and the healthy controls did not. Additionally, the lack of a trauma-exposed control group limits the ability to differentiate the effects of trauma per se versus phenomena specific to PTSD.

1.2.5. Receptor Studies

Several neurotransmitters are implicated in the regulation of processes of emotional experiencing and responding, including gamma-amino-butyric acid (GABA), opioids, and serotonin. GABA is a leader among neurotransmitters of interest in anxiety. Evidence from animal models of stress, the known anxiolytic effects of GABA-A receptor-modulating benzodiazepines in humans, and the dense localization of these receptors in emotional regulation regions such as the paralimbic PFC, amygdala, and hippocampus points to the role of GABA in anxiety. Two studies have used [^{123} I]-iomazenil (IMZ) SPECT to investigate central benzodiazepine receptors in PTSD. Bremner et al. (2000) reported a 41% reduction in distribution volume (DV) of [^{123} I]-IMZ in the MPFC (BA9) in 13 Vietnam veterans with combat-related PTSD compared with 13 case-matched healthy subjects. General psychopathological symptom scores on the Brief Psychiatric Rating Scale significantly positively correlated with receptor binding in BA9 in the PTSD subjects. A recent study comparing regional total DV between 19 Gulf War veterans with PTSD and 19 age-matched, healthy, nondeployed veterans found no between-group regional difference in BZ receptor binding (with or without global normalization) (Fujita et al. 2004). In the PTSD group, the normalized regional total DV (V_T') in the right superior temporal gyrus showed a significant negative correlation with childhood trauma scores, the significance of which is unclear. The presence of significant current comorbid mood and anxiety disorders and differences in illness duration between the

PTSD populations across studies may have contributed to the disparate findings. Additionally, the absence of a trauma-exposed control group limits the interpretation of the findings' specificity.

Serotonin (or 5-hydroxytryptamine, 5-HT) systems are modulatory with regard to other neurotransmitter systems that are relevant in anxiety and stress responses. The therapeutic response to selective serotonin reuptake inhibitors (SSRIs) in PTSD suggests their role in the disorder. The effect of SSRIs is possibly in part mediated by changes in the auto receptor 5-HT_{1A}, which is present in the prefrontal cortex. One group investigated 5-HT_{1A} receptor binding in 12 unmedicated patients with PTSD compared with 11 never-traumatized, healthy comparison subjects (predominantly women in both groups) and did not find any between-group difference in receptor binding (Bonne et al. 2005). The authors speculated whether the heterogeneity of the PTSD group (with regard to trauma type and duration) may have contributed to this negative finding.

The endogenous opioid system is another neuromodulatory system implicated in fear behavior, regulation of affective states, and suppression of affective defensive behavior (Good and Westbrook 1995; Shaikh et al. 1991; Zubieta et al. 2003). Although these processes appear relevant to the manifestations of PTSD, and abnormalities of the opioidergic system have been suggested in PTSD (Pitman et al. 1990b), surprisingly little attention has been paid to the opioid system in neurobiological or psychopharmacological research into PTSD. We recently completed a study examining carfentanil binding (a μ opioid receptor-selective ligand) in PTSD. The study involved PTSD, trauma exposed, non-PTSD, and healthy control groups. We found differential binding in the ACC and amygdala in the PTSD group relative to the control groups (Liberzon et al. submitted).

2. Summary and Future Directions

Several components of cognitive-emotional (e.g., threat related), processing including perception of fearful stimuli, experience of anxiety and fear, fear conditioning, emotional memory, extinction, and habituation appear to have specific relevance to the symptomatology and mechanisms of PTSD. Structures such as the medial prefrontal cortex, the rostral and subgenual ACC, insula, extended amygdala, hippocampus, subregions of the thalamus, and related sensory cortical areas appear to subserve threat-related processing, and the experience and expression of anxiety (LeDoux 2000; Phan et al. 2002, 2004b). Several of the same regions identified in animal and human studies of threat-related processing and self-related and socially related processing appear to be involved in the symptoms and functional correlates of PTSD, as evidenced in neuroimaging findings.

Accumulating evidence in PTSD points to regionally specific, impaired blood flow patterns that suggest reduced function in the medial PFC and anterior cingulate regions (Bremner et al. 1999b; Shin et al. 1999, 2001; Lanius et al. 2001), and increased/altered blood flow patterns suggesting increased responsivity of the ex-

tended amygdala and insula regions (Rauch et al. 1996, 2000; Liberzon et al. 1999b). It is important to note that these findings are not always consistent across studies and must be considered open to further exploration. Several methodological issues may contribute to these inconsistencies that need to be addressed in future studies. Caution is to be exercised when interpreting findings from neuroimaging studies that involve small sample sizes, heterogeneous populations (age, sex, type and severity of trauma, symptom subtypes, presence of comorbid conditions, and substance histories), and varying methodologies that limit broad generalization, as well as from unreplicated studies. Preliminary studies suggest that some of the changes observed in functional studies may relate to changes in neuroreceptor functioning of GABA, opioid, and 5-HT-related receptor systems. Thus, while the past two decades have witnessed a parallel progression of several lines of evidence, all of which enhance the understanding of some aspects of the PTSD state, clearly much work remains to be done. The exact relationships between structure and function, and between pathophysiology and symptomatology, remain unknown.

On the other hand, imaging studies that are beginning to integrate different lines of inquiry, including genetic, neurochemical/receptor, HPA axis, and blood flow parameters, are appearing in the literature. Such studies may provide meaningful information in complex neuropsychiatric conditions such as PTSD. This research holds the exciting promise of helping to identify neurobiological factors that may confer vulnerability or resilience to PTSD, helping to delineate risk factors from the effects of trauma and the effects of the disorder per se, and offering meaningful clues to the pathophysiology of PTSD. This progress will be essential for the future development of effective prevention and treatment strategies for this disorder.

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Functional Abnormality of the Prefrontal Cortex in Posttraumatic Stress Disorder: Psychophysiology and Treatment Studies Assessed by Near-Infrared Spectroscopy

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1. Introduction

A growing number of functional neuroimaging studies on posttraumatic stress disorder (PTSD) have described the abnormal response of amygdala and prefrontal cortex to traumatic stimuli (Bremner et al. 1999; Shin et al. 2001; 2004; Lanius 2001; Zubieta et al. 1999; Matsuo et al. 2003a). Furthermore, it has been reported that these abnormalities respond to treatment (Levin et al. 1999; Seedat et al. 2004; Carey et al. 2004). A large number of the studies on the prefrontal cortex in PTSD have shown diminished activation of this region in response to trauma-related stimuli (Bremner et al. 1999; Shin et al. 2001, 2004; Lanius et al. 2001). In contrast, several studies have shown greater activation of the prefrontal cortex (Zubieta et al. 1999; Matsuo et al. 2003a). In a positron emission tomography (PET) study (Bremner et al. 1999), combat veterans with PTSD showed diminished activation of the medial prefrontal cortex in response to trauma-related stimuli. Moreover, the decreased blood flow in the medial prefrontal cortex was found to be negatively correlated with increased blood flow in the amygdala in response to the traumatic stimuli and positively correlated with the severity of the symptoms. This suggested that insufficient suppression of the medial prefrontal cortex in response to the overactivation of the amygdala could be considered as a pathophysiological model of PTSD (Shin et al. 2004). Functional magnetic resonance imaging (fMRI) studies have shown similar findings (Lanius et al. 2001; Shin et al. 2001). Using single photon emission computed tomography (SPECT), Zubieta et al. (1999) examined regional cerebral blood flow (rCBF) responses to a combat stress-related auditory stimulus in Vietnam war veterans with PTSD and found that PTSD patients showed a significant increase in blood flow to the medial prefrontal cortex, which correlated at trend

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levels with psychophysical measures of stress response. In summary, abnormal activation of some parts of the prefrontal cortex might possibly be involved in the pathophysiology of PTSD.

2. Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) is a new functional neuroimaging method that uses near-infrared light. NIRS can monitor relative changes in oxygenated [oxyHb] and deoxygenated [deoxyHb] hemoglobin in certain activated regions (Jobsis 1977). Near-infrared light, the intensity of which is modulated at different frequencies at two wavelengths at approximately 800 nm, is combined by a beam coupler and directed to the surface of the scalp by a flexible fiber. During its course from the injector to the detector, a portion of the near-infrared light is absorbed by hemoglobin (Watanabe et al. 1996). The Beer-Lambert law is used to calculate the changes in [oxyHb] and [deoxyHb] based on the absorption characteristics. NIRS can measure a banana-shaped area at a depth of 0.9 to 1.3 cm from the brain surface, although determining the precise area of the brain by NIRS is difficult because it has a low spatial resolution (Gratton et al. 1994; Koizumi et al. 1999). When a certain brain region is activated, blood vessels are dilated, and the regional cerebral blood volume increases. The increase in cerebral blood volume is larger than that of the oxygen consumption in the activated region (Gsell et al. 2000). Dilatation of blood vessels also results in an increase in blood flow velocity. The increased blood flow efficiently flushes [deoxyHb] from the activated brain region. As a result, cognitive activation can be detected by NIRS as an increase in [oxyHb] accompanied by a decrease in [deoxyHb] (Strangman et al. 2002).

NIRS offers certain advantages in studying patients with psychiatric disorders. NIRS can measure successive changes in cerebral oxygenation and hemodynamic response that accompany those in neuronal activity in real time due to its high temporal resolution (0.1–1.0 s). When compared with PET and MRI, NIRS is a noninvasive procedure that can be performed at the bedside with the patient in a normal position because the device is smaller and easy to carry. Moreover, NIRS is useful for evaluating subjects who may move in response to emotional stimuli, because NIRS is relatively insensitive to motion artifacts.

Using NIRS, some evidence regarding activation of frontal cortex has been accumulated. Increase in [oxyHb] during frontal tasks in healthy volunteers (Hock et al. 1995; Villringer et al. 1997; Herrmann et al. 2003; Watanabe et al. 2003) and reduced prefrontal activation in patients with schizophrenia or mood disorders have been successfully detected by NIRS (Okada et al. 1996; Fallgatter et al. 2000; Matsuo et al. 2000, 2002, 2004; Herrmann et al. 2004; Shinba et al. 2004; Suto et al. 2004). For instance, Matsuo et al. (2005) have examined prefrontal activation in late-onset depressive patients and in control subjects during a cognitive task and carbon dioxide inhalation. They found that activation of the prefrontal cortex during the cognitive task was significantly less in the patients with depression than in the controls, although task performance was not significantly different between the

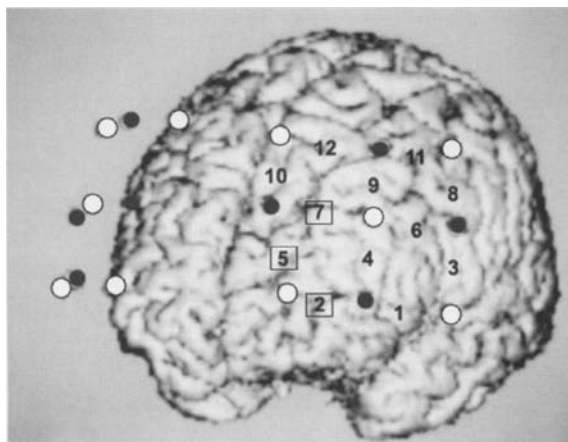


Fig. 1. Positions of the near-infrared spectroscopy (NIRS) probes and the area measured shown by three-dimensional magnetic resonance imaging. *Open circle*, injector point; *closed circle*, detector point; *number*, measurement point. Channels 1 to 7 in the left prefrontal cortex and the corresponding seven probes in the right were examined. During trauma-related stimuli, oxygenated hemoglobin (oxyHb) was increased in channels 2, 5, and 7 in subjects with posttraumatic stress disorder (PTSD) compared with the controls, although differences were not significant ($P < 0.10$). The *number* of the activated channel is surrounded by a *square*. This figure depicts the location of the channels 2, 5, and 7. The locations of other channels do not correctly represent the measurement area, because the circles and numbers show the locations on the skin, but not on the brain. (From Matsuo et al. 2003a, with permission)

two groups. Furthermore, the vasomotor reactivity to carbon dioxide inhalation was significantly lower in the patients with depression than in the controls; this suggested that prefrontal microvascular dysregulation is involved in the pathophysiology of functional hypofrontality in late-onset depression. These previous studies indicate that NIRS is a useful method for investigating functional brain mechanisms in psychiatric diseases.

3. NIRS Studies in PTSD

Matsuo et al. (2003b) studied the relationship between hemodynamic response in prefrontal cortex during a cognitive task and memory function, based on previous neuropsychological studies showing that subjects with PTSD have cognitive impairments (Koenen et al. 2001; Brandes et al. 2002). The study included 34 victims of the Tokyo subway sarin attack (8 subjects met the PTSD criteria and 26 did not); changes in [oxyHb] and [deoxyHb] occurring in their prefrontal cortex during a

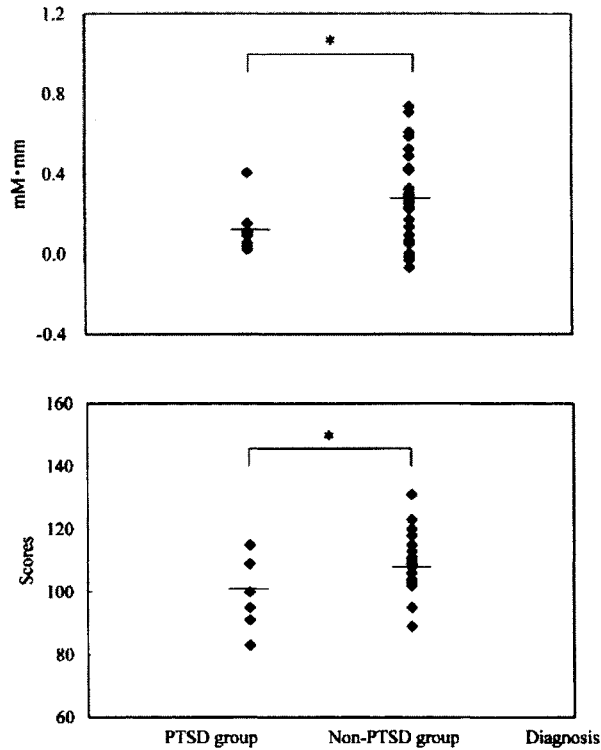


Fig. 2. *Upper*, alterations in oxyHb during the Verbal Fluency Test ($t = -7.4$; asterisk, $P < 0.01$). *Abscissa*, diagnosis; *ordinate*, alteration of oxyHb. The unit of the alteration was molar concentration multiplied by length (mM·mm). *Lower*, distribution of scores of attention and concentration on the WMS-R ($t = -2.2$; asterisk, $P = 0.03$). *Abscissa*, diagnosis; *ordinate*, scores of attention and concentration in the WMS-R. (From Matsuo et al. 2003b, with permission)

verbal fluency task and a control task were quantified. The area measured was approximately prefrontal cortex (Fig. 1). During the verbal fluency task, the typical time course of [oxyHb] increased and that of [deoxyHb] decreased. Furthermore, the responses of [oxyHb] and total hemoglobin ([total Hb] = [oxyHb] + [deoxyHb]) in the subjects diagnosed with PTSD were significantly lower than in those without PTSD (Fig. 2 *upper*), although no significant difference was observed in the task performance between the two groups. Mean “attention and concentration” scores on the Wechsler Memory Scale-revised (WMS-R) in the subjects with PTSD were significantly lower than those in the subjects without PTSD (Fig. 2 *bottom*); these scores were positively correlated with increase in [total Hb] during the verbal fluency task. Based on these results, it was suggested that the “frontal dysfunction” observed in the subjects with PTSD might be a phenomenon that is secondary to reduced attention capacity. Furthermore, these investigators examined the hemody-

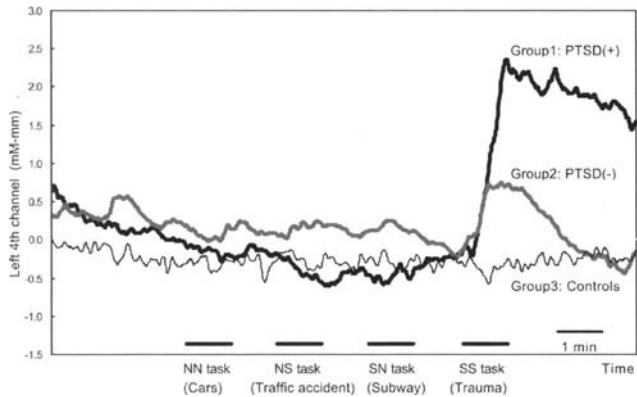


Fig. 3. Typical time courses of the NIRS variables in oxyHb of left fourth channel during four target images in a victim with PTSD (*Group 1*), a victim without PTSD (*Group 2*), and a control subject (*Group 3*). Time resolution was 1 s. *Abscissa*, time (min); *ordinate*, hemoglobin concentration (mM mm). In this NIRS instrument (ETG-100), hemoglobin levels are expressed as the product of hemoglobin concentration and optical path length. *NN*, nonspecific and nonstimulating image; *NS*, nonspecific and stimulating image; *SN*, specific and nonstimulating image; *SS*, specific and stimulating (trauma) image. (From Matsuo et al., 2003a, with permission)

dynamic responses produced in the prefrontal cortex by trauma-related images in the same victims and 12 nonvictims (Matsuo et al. 2003a). The task used four types of video images: nonspecific and nonstimulating image (*NN*; cars driving on an urban highway), nonspecific and stimulating image (*NS*; traffic accidents), specific and nonstimulating image (*SN*; subway running toward a platform), and specific and stimulating (trauma) image (*SS*; the image showing the victims at a subway station at the time of the Tokyo subway sarin attack in 1995). Hemodynamic response in the prefrontal cortex was monitored during the viewing of these images. Skin conductance response (*SCR*) was also examined. During viewing of the trauma-related images, changes in [oxyHb] were found to be significantly increased in the victims with or without PTSD, whereas changes in [deoxyHb] were significantly decreased only in victims with PTSD (Fig. 3). No significant changes were observed in [oxyHb] and [deoxyHb] in the control subjects. Significantly enhanced *SCR* was also observed in response to trauma-related stimuli in the victims with PTSD. These results suggest that the subjects with a history of this traumatic experience showed an increased activity in the prefrontal cortex in response to trauma-related stimuli, and that NIRS results were associated with psychophysiological responses in PTSD.

4. Neuroimaging Studies of Treatment in PTSD

4.1. *Selective-Serotonin Reuptake Inhibitor*

Few neuroimaging studies have been conducted on the effects of treatment in PTSD. In a structural MRI study, Vermetten et al. (2003) examined the association between long-term treatment with a selective serotonin reuptake inhibitor (SSRI) and hippocampal volume in PTSD. These investigators found a significant improvement in PTSD symptoms and verbal memory accompanied by an increase in hippocampal volume after treatment for 9–12 months. In a study that used SPECT with Tc-99m HMPAO, the association between the effect of pharmacological treatment and the change in rCBF was investigated (Seedat et al. 2004; Carey et al. 2004). The rCBF of the patients with PTSD was measured prior to and 8 weeks after treatment with citalopram, an SSRI. The rCBF in the left medial temporal cortex in the posttreatment patients was significantly decreased when compared with that in the pretreatment patients. Additionally, a significant correlation was observed between reduction in Clinician-Administered PTSD Scale (CAPS; Blake et al. 1995) scores and activation in the left paracingulate region (medial prefrontal cortex) in the posttreatment patients (Seedat et al. 2004).

4.2. *Eye Movement Desensitization and Reprocessing*

Several other studies have been conducted on treatments by eye movement desensitization and reprocessing (EMDR) and changes in rCBF. EMDR, introduced by Shapiro, is a relatively new psychological treatment for PTSD (Shapiro 1989, 1995, 2001). In EMDR therapy, clients are instructed to recall a representative image, body sensation, and negative cognition of their traumatic experience along with eye movements. Clients are instructed to move their eyes by following fingers that are rapidly moved from side to side in front of them. Based on the clients' feedback, their traumatic memories are rendered nondistressful by repeating memory recall with eye movement. The procedure also involves a cognitive component by using a positive cognition associated with the traumatic experience. After completing the desensitization, the repetitions of the eye movement are used to reinforce and "install" the positive cognition. The effectiveness of EMDR in the treatment of trauma-related disorders has been reported by a variety of case studies and clinical practices (Wolpe and Abrams 1991; Lipke and Botkin 1993), numerous controlled studies (Forbes et al. 1994; Wilson et al. 1994), and a meta-analysis (Van Etten and Taylor 1998).

In a study on the association between the effect of EMDR and brain function, Lamprecht et al. (2004) described a change in event-related brain potentials (ERPs) produced by EMDR in PTSD clients. They used a modified oddball paradigm containing auditory standard, target, and novel tones and assessed the ERPs before and after EMDR. Psychometric assessments showed a significant improvement in PTSD

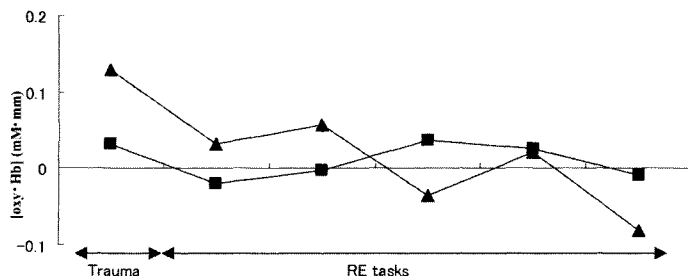


Fig. 4. Typical changes in oxyHb in the prefrontal cortex during the tasks of trauma-related memory recall with eye movement prior to eye movement desensitization and reprocessing (EMDR) treatment. *Triangles* show changes in [oxyHb] in an EMDR responder; *squares* show changes in [oxyHb] in an EMDR nonresponder. *Trauma* refers to traumatic memory recall, and *RE tasks* refer to trauma-related memory recall with eye movement. The [oxyHb] in the responder was decreased by RE tasks, while that in the nonresponder was unchanged

symptoms after EMDR treatment. The ERPs of the clients showed a significant reduction in the P3a amplitude in the posttreatment recording when compared with that of the control group receiving the sham treatment. The results suggested that the PTSD clients were less prone to distraction and intrusions by irrelevant stimuli. This is because the P3a component has been associated with the evaluation of novel events (Friedman et al. 2001) and interpreted as an index of automatic allocation of attention toward a source of stimulation (Ford et al. 1976). PTSD clients' orientation to novel stimuli and arousal level were reduced after the EMDR treatment. Levin et al. (1999) presented a case report on the change in brain activation during traumatic memory recall before and after EMDR using SPECT. The activation of the left frontal lobe and anterior cingulate gyrus were increased to a greater extent during traumatic memory recall after EMDR treatment when compared with that prior to EMDR treatment. They considered that successful EMDR treatment enhances the ability to differentiate real from imaginary threats. Based on these investigations, EMDR may have an influence on the brain function as well as SSRIs. In order to further elucidate the relationship between emotional memory and eye movement, Ohtani et al. (2005) investigated changes in [oxyHb] concentrations in prefrontal cortex in ten healthy subjects using NIRS during a task that involved emotional recall with or without eye movements. Six subjects experienced unpleasant feelings and demonstrated a significant increase in [oxyHb] during the emotional recall. The increase in [oxyHb] was smaller during the emotional recall with repetitive eye movements. Based upon these results, they suggested that eye movements might reduce the hemodynamic response of prefrontal cortex to emotional memory recall. Furthermore, Ohtani et al. (manuscript in preparation) examined the hemodynamic response of the prefrontal cortex during trauma-related memory recall with eye movements in PTSD clients treated with EMDR. The preliminary data showed that [oxyHb] in the prefrontal cortex significantly decreased during the

trauma-related memory recall with repetitive eye movements in one session in the EMDR responders (Fig. 4). The [oxyHb] concentration significantly decreased further during the memory recall as these sessions were repeated. These results might act as evidence for the biological mechanisms of EMDR.

Previous neuroimaging studies have identified functional abnormalities in the prefrontal cortex of individuals with PTSD. These abnormalities have been observed to respond to certain treatments, including EMDR. Thus, the prefrontal cortex might be involved in the pathophysiology of PTSD and play an important role in the alleviation of PTSD symptoms. Additionally, NIRS may play a valuable role in the assessment of PTSD, because it is noninvasive and sensitive to real-time functional change in the prefrontal cortex. NIRS is a promising technology, and, following further evaluation, might be a useful tool for monitoring treatment outcomes in PTSD.

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Neural Correlates of Symptom Improvement in Posttraumatic Stress Disorder: Positron Emission Tomography Study

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1. Introduction

Memory functioning and its disorder are central to understanding posttraumatic stress disorder (PTSD). The intrusive recall of memories for the traumatic event, or reexperiencing, is a central feature of PTSD (Clark et al. 2003). In those suffering from this symptom, the past traumatic experience is recalled intrusively and without intent, including in nightmares, with a sense that the traumatic event is occurring right now, accompanied by emotional response such as fear, and physiological responses such as palpitation and dyspnea.

Several neuroimaging studies with provocation of PTSD symptoms have been reported, in which reexperiencing was provoked using personalized trauma scripts or trauma-related sounds or pictures (Rauch et al. 1996; Shin et al. 1997, Bremner et al. 1999). These studies have suggested abnormalities in some brain regions such as the amygdala, Broca's area, and the anterior cingulate cortex (ACC) in patients with PTSD. However, few studies have investigated changes in neural activities during provocation of reexperiencing before and then after reported improvement of PTSD.

We studied the neural basis of reexperiencing in patients with PTSD using positron emission tomography (PET) and investigated the change in the neural activities from before to after improvement of PTSD symptoms. As a therapy for PTSD, we used the eye movement desensitization and reprocessing (EMDR) procedure. Al-

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though the mechanism by which EMDR acts is unclear, its efficacy for PTSD is comparable to cognitive behavioral therapy (Davidson and Parker 2001).

2. Methods

2.1. *Participants*

A man and four women aged 26–38 years, all right-handed, participated in the study. All were victims of criminal incidents, such as attempted murder or physical assault. They had no history of neurological or psychiatric disease before their traumatic experiences, which took place after their adolescence. When they were referred to our hospital, they had already been given medications including serotonin-selective reuptake inhibitors (SSRI) for at least 2 months. At our first examination, they had various symptoms specific and unspecific to PTSD, such as reexperiencing, depression, anxiety, insomnia, and irritation. We continued medication for at least 12 weeks, but their symptoms were unimproved. At that point, they were examined for the diagnosis of PTSD using the clinician-administered PTSD scale (CAPS; Blake et al. 1995) and impact of event scale-revised (IES-R; Weiss and Marmar 1997). The CAPS and IES-R scores of all participants were very high (described below in Results), and we therefore considered that they all met the criteria of PTSD.

The present study was approved by the Ethical Committee of The Graduate School of Medicine, Osaka University. All patients gave their written informed consent for participation.

2.2. *Eye Movement Desensitization and Reprocessing*

All patients underwent eye movement desensitization and reprocessing (EMDR) sessions while taking a constant dose of SSRI. To avoid any technical faults in the EMDR procedure, a proficient psychiatrist, who was an expert in EMDR, performed the EMDR sessions for all patients. Each patient underwent an EMDR session once every 1 or 2 weeks. The rough criteria for completing the EMDR sessions were defined by the specific indices of the EMDR procedures (Lohr et al. 1992). When the subjective units of disturbance scale (SUDs; range 1–10) became less than 3 points, and the validity of cognition scale (VoC; range 1–7) became more than 5 points, and this state had been maintained for about 4 weeks, we considered that the symptoms of PTSD had improved. Any patients who did not meet these criteria after ten EMDR sessions were excluded. Before and after the series of EMDR sessions, patients underwent identical PET scanning procedures as described below, accompanied by symptom assessments with CAPS and IES-R.

2.3. PET Activation: Provocation of Reexperiencing

Imagery of the traumatic experience using the patients' personalized scripts was used to provoke reexperiencing (Bremner et al. 1999; Lanius et al. 2002). Each participant underwent six PET scannings. During each PET scanning, one of two types of auditory scripts recorded aloud by an examiner was presented as a stimulus. The participants were required to close their eyes and listen to the auditory stimuli, then to remember the scene described in the scripts. The duration of each presentation was about 70 s. In the Traumatic condition, the script written in the first person was a description of his/her individual traumatic experience that most likely caused the PTSD. In the Neutral condition, the script depicted an emotionally neutral experience, also written in the first person. Each condition was repeated three times for each participant, during which the same script was used repeatedly. The order of two conditions was pseudorandom and counterbalanced among the patients.

Through the experiment, participants' heart rate was recorded. Immediately after each script presentation, patients were required to rate verbally their subjective degree of fear using a 100-point visual analog scale (VAS), in which 0 indicated no fear and 100 points indicated maximal fear in their life. If rate or VAS increased markedly after presentation of the stimuli, especially in the *Traumatic* condition, the participants were given rest until their heart rates or VAS returned to the calm level to confirm that the physical reaction or fear feeling would not persist in the next task.

2.3.1. PET Data Acquisition

Regional cerebral blood flow (rCBF) data were acquired using a Headtome V scanner (Shimadzu, Kyoto) in a three-dimensional (3D) mode with a shield to block the radiation from the participant's trunk. Attenuation by the tissues of the head was corrected using a transmission scan obtained prior to the emission scans for each participant. Tracer administrations were started simultaneously with each task. A dose of 296 MBq [^{15}O]-H₂O was administered by a semibolus injection. After the tracer appeared in the brain (about 40 s after the beginning of each administration), scanning was started and continued for 90 s. The images reconstructed using filtered back projection were regarded as estimates of rCBF.

2.3.2. PET Data Analysis

Statistical parametric mapping (SPM) software (SPM99; Wellcome Department of Cognitive Neurology, London) implemented in Matlab (Mathworks, Sherborn, MA, USA) was used to analyze the PET images. Each image was realigned to the first emission scan for each participant in order to remove any motion artifacts and was spatially normalized to the standard stereotactic space. After normalization, the images were smoothed with a 16-mm full-width at half-maximum Gaussian filter to increase the signal-to-noise ratio. The resultant set of voxel values for each contrast

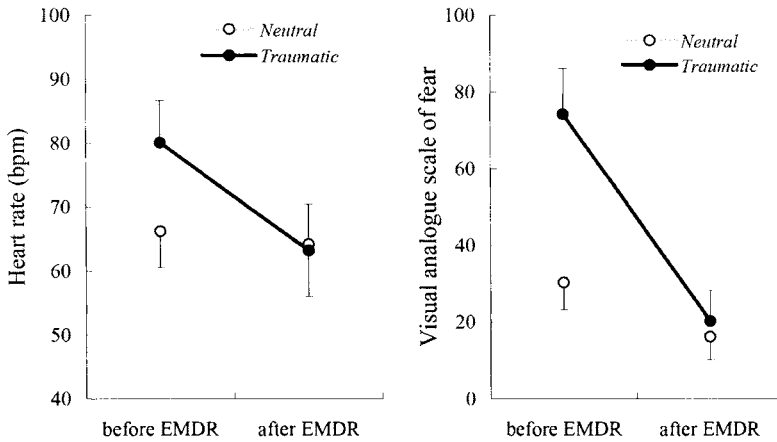


Fig. 1. Comparison of heart rates (*left*) and score of the visual analogue scale (VAS) of fear (*right*) for the *Traumatic* and *Neutral* conditions before and after eye movement desensitization and reprocessing (EMDR)

constituted a *t*-statistic SPM $\{t\}$ (Friston et al. 1994). The identical PET scan procedure was conducted both before and after the EMDR sessions.

3. Results

3.1. Behavioral Results

The indices of PTSD symptoms decreased remarkably after the EMDR sessions in all patients. The mean CAPS score (standard deviation) changed from 92.4 (18.9) before EMDR to 35.4 (11.0) after EMDR. The mean IES-R score changed from 62.0 (13.2) before EMDR to 17.6 (8.1) after EMDR. These changes indicated that PTSD symptoms improved with EMDR.

During PET scans in the *Traumatic* condition before the EMDR sessions, all patients reported that they recalled their experiences vividly as if the traumatic event were occurring right at the time, and they often had feelings of dyspnea or palpitations as well as fear. Two of the patients shed tears, and another one showed dissociative symptoms, because they could not reply to calling by the examiners for several seconds. During the PET scans after the EMDR sessions, no patients showed these emotional responses.

Heart rate increased remarkably in the *Traumatic* condition as compared with the *Neutral* condition before EMDR, while no difference between the two conditions was seen after EMDR (Fig. 1). The condition (*Traumatic* and *Neutral*) by time (before and after EMDR) interaction was significant ($P < 0.01$). The VAS scores of subjective fear were higher in the *Traumatic* condition than in the *Neutral* condition before EMDR. The VAS score in the *Traumatic* condition decreased remarkably after EMDR, so that the difference between these two conditions became minimal af-

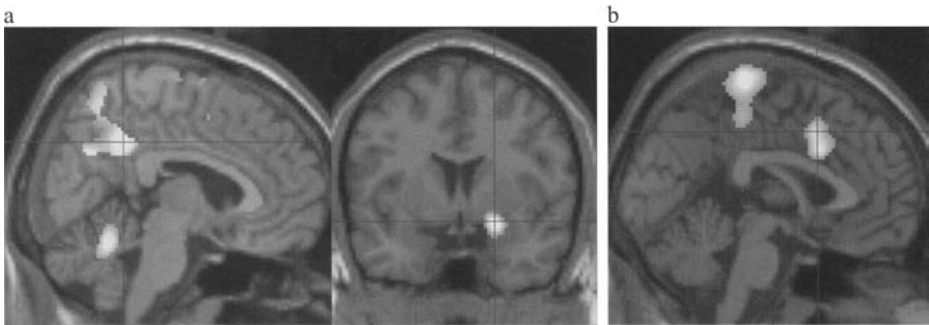


Fig. 2. **a** Brain regions activated significantly in the *Traumatic* condition compared with the *Neutral* condition before EMDR sessions. **b** Brain regions deactivated significantly in the *Traumatic* condition compared with the *Neutral* condition before EMDR sessions

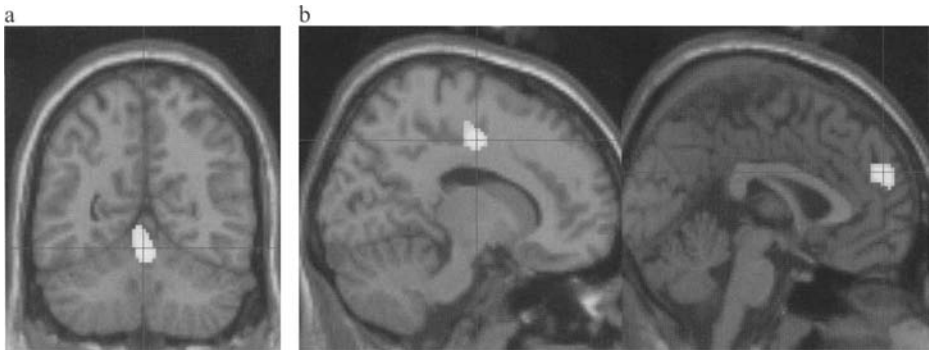


Fig. 3. **a** A brain region activated in the *Traumatic* condition before EMDR sessions significantly more than after the EMDR sessions. **b** Brain regions activated in the *Traumatic* condition after EMDR sessions significantly more than before the EMDR sessions

ter EMDR (Fig. 1). The condition by time interaction was significant ($P < 0.005$).

3.2. PET Results

Before the EMDR sessions, in the *Traumatic* condition compared with the *Neutral* condition, the PET data showed significant activations in the right posterior cingulate cortex [Montreal Neurological Institute (MNI) coordinates $x, y, z = +3, -49, +30$; Brodmann area (BA) 31] and the vermis ($x, y, z = 0, -55, -12$) (Fig. 2a), and the right amygdala ($x, y, z = +20, -2, -10$) (Fig. 2b left) ($P < 0.05$, corrected for whole brain). The PET data showed significant deactivations in the *Traumatic* condition compared with the *Neutral* condition in the left ACC ($x, y, z = -2, +12, +42$; BA32/24) and the bilateral parietal cortices (Fig. 2b right) ($P < 0.05$, corrected). After the EMDR sessions, no significant activations were seen.

A comparison between before and after EMDR sessions, i.e., (*Traumatic* – *Neu-*

tral condition after EMDR) versus (*Traumatic* – *Neutral* condition before EMDR), was made using a double subtraction procedure (condition by time interaction). This procedure revealed that the activation in the vermis ($x, y, z = 0, -56, -14$) in the *Traumatic* condition before EMDR was significantly greater than after EMDR (Fig. 3a) ($P < 0.05$, corrected). In addition, activations in the region slightly posterior to the right ACC ($x, y, z = +14, -10, +44$; BA31) (Fig. 3b *left*) and in the medial frontal cortex ($x, y, z = 0, +54, +26$; BA9) (Fig. 3b *right*) were significantly greater after EMDR than before EMDR ($P < 0.05$, corrected).

4. Discussion

We considered that reexperiencing could be provoked in the PTSD patients by the script-driven imagery procedure in the present study. This interpretation was supported by the remarkable increases in the heart rate and higher VAS score of fear in the *Traumatic* condition before the EMDR sessions. The symptoms of PTSD that persisted despite administration of SSRIs improved obviously after EMDR, implying that the improvement should be attributed to EMDR or combination of EMDR and medications.

The amygdala activated in the *Traumatic* condition as compared with the *Neutral* condition before EMDR in this study is well known to be related to emotions such as fear (Breiter et al. 1996; Morris et al. 1996). In addition, the amygdala is involved in fear conditioning, which is one of the models of PTSD pathology such as reexperiencing symptoms (Doronbekov et al. 2005). The right posterior cingulate cortex activated in the *Traumatic* condition before EMDR has afferent inputs indirectly from the amygdaloid complex and strong efferent connection to the parahippocampal and entorhinal cortices. Therefore, this area is assumed to mediate an interaction between emotion and memory (Maddock 1999). The symptom of reexperiencing is likely to correlate with activation of the posterior cingulate cortex, because reexperiencing could be considered as an emotional response to the past memory.

The vermis has been shown to be involved in fear-conditioning consolidation in some animal studies (Sacchetti et al. 2002). Given that the vermis was activated during reexperiencing in the present study, it may be that repetition of reexperiencing makes the fear conditioning more consolidated. It may be a mechanism by which the patients with PTSD cannot become habituated to the reexperiencing symptom in spite of its repetition. Furthermore, it is speculated that EMDR could inhibit the function of the vermis, that is, fear-conditioning consolidation, resulting in improvement of reexperiencing.

Although the regions mentioned here were activated in the *Traumatic* condition before EMDR, these regions were not activated in the *Traumatic* condition when the symptom improved after EMDR. This result also suggests that these areas are involved in developing reexperiencing.

On the other hand, the left ACC was significantly deactivated in the *Traumatic* condition before EMDR, and the activation in the region slightly posterior to the

right ACC after EMDR was significantly greater than that before EMDR. If laterality is not taken into account, the activities in the cingulate cortex and the amygdala seem to change reciprocally. In previous studies (Bremner et al. 1999; Pitman et al. 2001), it was hypothesized that in PTSD the ACC might fail to inhibit the amygdala, which could result in the reexperiencing symptoms. This hypothesis is consistent with our results in the present study. It is suggested that deactivation of amygdala, and perhaps improvement of reexperiencing, might depend upon increased activity in the cingulate cortex.

However, the causes of reduction of the ACC activity in PTSD are still unclear. Altered ACC activity may be merely diaschisis or the consequences of other essential lesions. On the other hand, the ACC may be the essential region for development of PTSD. A clinical study using magnetic resonance imaging and voxel-based morphometry reported that the volume reduction of gray matter in the left ACC significantly correlated with the severity of PTSD (Yamasue et al. 2003). If this volume reduction confers vulnerability for developing PTSD, the increase of blood flow in the right cingulate cortex seen after EMDR in our results could not be regarded as the effect of a fundamental cure. Rather, it may be considered that the increased activity in the right cingulate cortex is a compensation for the reduction in the left ACC function, such as inhibition of the amygdala. This compensation might be one of the targets on which the therapies of PTSD such as EMDR act.

The limitations of the present study include the absence of control groups of healthy participants or patients with other psychiatric diseases such as panic disorder. Accordingly, it is unclear whether the findings of our present study are specific to PTSD. Another limitation is the heterogeneity in the sex of the participants and in the type of their traumatic incidents. In many previous studies, examination of combat veterans allowed the type of patients' traumatic incidents to be almost homogeneous. On the other hand, in Japan, the patients with PTSD we encounter in the clinical practice are mostly isolated cases such as criminal victims. Consequently, we believe that investigating the common neural basis underlying these heterogeneous PTSD cases due to various traumatic etiologies is also important.

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Cognitive Behavior Therapy: Implications from Advances in Neuroscience

Richard A. Bryant

1. Introduction

There is prevailing opinion that cognitive behavior therapy (CBT) is the treatment of choice for posttraumatic stress disorder (PTSD). Despite its marked success in reducing PTSD symptoms, there are significant challenges ahead in making CBT more effective in reducing PTSD symptoms in affected people. This chapter reviews the current evidence for CBT for PTSD and considers the interventions currently available in the context of recent advances in neuroscience. In particular, this chapter conceptualizes PTSD as a form of fear conditioning, and it considers the evidence for adaptation and successful therapy after trauma as forms of extinction learning. In this context, this chapter considers options for enhancing CBT through neural facilitation of learning.

2. What is Cognitive Behavior Therapy?

CBT typically involves several core components. These usually include education, anxiety management, cognitive therapy, and exposure. Education components provide information about common symptoms following a traumatic event. This education highlights the hallmark symptoms of the posttrauma response and discusses the way in which the core symptoms will be treated during the course of therapy. The aim is to legitimize the trauma reactions, to help the patient develop a formulation of their symptoms, and to establish a rationale for treatment. Anxiety management techniques aim to provide individuals with coping skills to assist them to gain a sense of mastery over their fear, to reduce arousal levels, and to assist the individual when engaging in exposure to the traumatic memories. Anxiety management approaches often include breathing retraining, muscle relaxation, and self-talk. Cognitive restructuring is included because of the importance of appraisals in the etiology and maintenance of PTSD (Ehlers and Clark 2000). Cognitive restructuring involves teaching patients to identify and evaluate the evidence for negative

automatic thoughts, as well as helping patients to evaluate their beliefs about the trauma, the self, the world, and the future. Prolonged imaginal exposure requires the individual with PTSD to vividly imagine the trauma for prolonged periods. The therapist assists the patient to provide a narrative of their traumatic experience in a way that emphasizes all relevant details, including sensory cues and affective responses. In an attempt to maximise the sense of reliving the experience, the individual may be asked to provide the narrative in the present tense, speak in the first person, and ensure that there is focus on the most distressing aspects. Prolonged exposure typically occurs for at least 45 min and is usually supplemented by daily homework exercises. Variants of imaginal exposure involve requiring clients to repeatedly write down detailed descriptions of the experience (Resick and Schnicke 1993) and implementing exposure with the assistance of virtual reality paradigms implemented via computer-generated imagery (Rothbaum et al. 2001). Many exposure treatments supplement imaginal exposure with *in vivo* exposure that involves live graded exposure to the feared trauma-related stimuli.

Possible mechanisms explaining the efficacy of exposure include promoting habituation of anxiety, correcting belief that anxiety persists unless avoidance occurs, promoting the incorporation of corrective information into the trauma memory, establishing that the trauma is a discrete event that is not indicative of the world being globally threatening, and enhancing self-mastery through management of the exposure exercise (see Jaycox and Foa 1996; Rothbaum and Mellman 2001). In any case, there is consensus on the conclusion that exposure is an effective treatment because it allows the individual to experience the memories and stimuli associated with the trauma in a safe manner in which they learn that these memories and cues are no longer threatening.

3. Does Cognitive Behavior Therapy Work?

There is now overwhelming evidence that CBT is an efficacious intervention for PTSD. Probably the first well-controlled CBT study for PTSD was conducted by Foa and colleagues, in which they randomly allocated female sexual assault survivors to prolonged exposure, stress inoculation training, supportive counseling, or a wait-list control group (Foa et al. 1991). This study provided participants with nine twice-weekly sessions and included blind assessments at both the posttreatment and 3-month follow-ups. Although stress inoculation training resulted in greater gains than supportive counseling or wait-list control at posttreatment, the prolonged exposure condition led to greater reduction in PTSD symptoms at follow-up. The authors interpreted these findings as indicating that whereas stress inoculation training led to short-term symptom reduction, prolonged exposure resulted in longer-term benefits because the fear networks were activated and modified.

Foa and colleagues subsequently replicated this study in a design that randomized 96 assault victims to prolonged exposure, stress inoculation training, the combination of prolonged exposure and stress inoculation training, and a wait-list con-

trol condition (Foa et al. 1999). The hypothesis tested was that the combination treatment would be superior to either of its components administered alone. The treatment was administered in nine individual sessions spaced twice weekly. The three active treatments were associated with reduced PTSD and depression relative to the wait-list condition. The intent-to-treat analysis indicated that prolonged exposure was superior to stress inoculation training and the combined treatment. That is, the main hypothesis of the study concerning the additive benefit of combined treatments was not supported. The authors suggested that this may have been because the session length was the same for all conditions, and this may have resulted in "information overload" (p. 199, Foa et al. 1999).

In a variant of CBT, Resick and colleagues have proposed cognitive processing therapy (CPT) as a structured combination of exposure and cognitive therapy based on five major cognitive themes that they suggest are central to rape victims' cognitive schema (Resick and Schnicke 1993). One of the features of their approach is that exposure is achieved through repeatedly writing the narrative. In a well-controlled study of Resick's cognitive processing therapy, Resick and colleagues compared CPT with exposure and a waiting minimal-attention condition (Resick et al. 2002). One hundred seventy-one female rape victims were randomized into one of the three conditions, and 121 completed treatment. The two active treatments consisted of 13 h of therapy conducted twice per week. Both intent-to-treat and completer analyses indicated that active treatments were highly efficacious and superior to the wait-list condition. There were no differences between the active treatments, except that CPT produced greater reduction on measures of guilt.

Subsequent well-controlled studies have attested to the efficacy of CBT with a variety of trauma populations, including survivors of motor vehicle accidents (MVAs), (Blanchard et al. 2003; Ehlers et al. 2003; Taylor et al. 2001) sexual assault (Foa et al. 1991, 1999), combat (Glynn et al. 1999), childhood sexual abuse (McDonough et al. 2005), and mixed trauma (Bryant et al. 2003a; Marks et al. 1998; Tarrier et al. 1999). Overall, these studies indicate that CBT markedly reduces PTSD and associated depressive symptoms, and that exposure appears to be the most efficacious component of treatment. Overall, combining cognitive therapy with exposure does not appear to result in any additive gains to the exposure therapy (Marks et al. 1998; Foa et al. 1999; see also Bryant et al. 2003).

A variant of CBT is eye movement desensitization and reprocessing (EMDR). EMDR has been proposed as a highly effective intervention that reduces the reexperiencing and anxiety features of PTSD. EMDR requires the patient to focus their attention on a traumatic memory while simultaneously visually tracking the therapist's finger as it is moved across their visual field and then to engage in restructuring of the memory (Shapiro 1995). A major difficulty with studies of EMDR is that many of them are flawed by significant methodological problems (for reviews, see Cahill et al. 1999; McNally 1999). In a recent review of 17 randomized clinical trials of the effectiveness of EMDR, Lohr et al. (1999) concluded that: (1) the effects of EMDR are based on self-report, (2) eye movements do not appear to be necessary for improvement, and (3) the effects observed suggest nonspecific effects or the effect of exposure alone (see also Cahill et al. 1999; McNally 1999). Overall,

EMDR does appear to be more effective than no treatment, supportive listening, and relaxation. In terms of EMDR's effectiveness relative to established CBT, one study randomly allocated civilian trauma survivors to nine sessions of either CBT or EMDR (Devilly and Spence 1999). Although this study found that both treatment groups improved at posttreatment, the gains made by participants who received CBT were greater than those made by participants who received EMDR. Furthermore, whereas those in the CBT condition maintained their treatment gains over the following 3 months, those in the EMDR group tended to relapse. The evidence against considering EMDR as a treatment of choice for PTSD is further supported by a recent 5-year follow-up of patients treated with EMDR (Macklin et al. 2000). This study found that treatment gains displayed initially after treatment did not maintain in this sample. The current status of EMDR is probably best summed up in the statement that "what is effective in EMDR (imaginal exposure) is not new, and what is new (eye movements) is not effective" (McNally 1999, p. 2).

4. Can Cognitive Behavior Therapy Prevent PTSD?

There have been attempts to prevent PTSD by administering CBT several weeks after trauma exposure. Foa et al. (1995) provided an abridged version of CBT several weeks after trauma. Each participant received four sessions and was then assessed by blind assessors at 2 months posttreatment and 5 months follow-up. At 2 months, whereas 10% of the CBT group met criteria for PTSD, 70% of a control group met criteria. It is possible that natural remission may have contributed to the results. Accordingly, Bryant and colleagues adopted a different approach by focusing on people shortly after trauma, who are high risk for PTSD development. Specifically, this approach focused on people with acute stress disorder (ASD), because prospective studies indicate that a majority of people with ASD will suffer long-term PTSD (for reviews, see Bryant 2003; Harvey and Bryant 2002). In an initial treatment study of ASD participants, Bryant and colleagues randomly allocated MVA or nonsexual assault survivors with ASD to either CBT or supportive counseling (Bryant et al. 1998). Both interventions consisted of five 1.5-h weekly individual therapy sessions. CBT included education about common posttraumatic reactions, relaxation training, imaginal exposure to the traumatic event, graded *in vivo* exposure, and cognitive restructuring. The supportive counseling condition included trauma education and more general problem-solving skills training in the context of an unconditionally supportive relationship. At the 6-month follow-up, there were fewer participants in the CBT group (20%) who met diagnostic criteria for PTSD compared with supportive counseling control participants (67%). In a subsequent study that dismantled the components of CBT, 45 civilian trauma survivors with ASD were randomly allocated to five sessions of either (1) CBT (prolonged exposure, cognitive therapy, anxiety management), (2) prolonged exposure combined with cognitive therapy, or (3) supportive counseling (Bryant et al. 1999). This study found that at the 6-month follow-up, PTSD was observed in approximately 20% of

both active treatment groups compared with 67% of those who received supportive counseling. In a subsequent study, this approach has demonstrated that CBT is effective in treating people with ASD after mild traumatic brain injury (Bryant et al. 2003b). In an attempt to improve the efficacy of CBT, another study randomly allocated civilian trauma survivors ($n = 89$) with ASD to either CBT, CBT plus hypnosis, or supportive counseling (Bryant et al. 2005a). The hypnosis component was provided immediately prior to imaginal exposure in an attempt to facilitate emotional processing of the trauma memories. This study added hypnosis to CBT because some commentators have argued that hypnosis may breach dissociative symptoms that characterize ASD (Spiegel 1996), and there is evidence that people with ASD are particularly hypnotizable (Bryant et al. 2001). In terms of treatment completers, more participants in the supportive counseling condition (57%) met criteria for PTSD at the 6-month follow-up than those in the CBT (21%) or CBT plus hypnosis (22%) condition. Interestingly, participants in the CBT plus hypnosis condition reported greater reduction of reexperiencing symptoms at posttreatment than those in the CBT condition. This finding suggests that hypnosis may facilitate treatment gains in ASD participants. Importantly, long-term follow-up assessments of these studies indicate that the treatment gains are maintained between 2 and 4 years after treatment (Bryant et al. 2003c; 2005b).

5. What is the Neural Basis of Adaptation to Trauma?

Recent developments in neuroscience have led clinicians to consider the role that neural activity plays in CBT. Theorizing about this issue can be traced back to earlier conceptualizations of the neural bases of response to a traumatic experience. Fear conditioning models of trauma response draw a comparison between the reactions of traumatized individuals and rats that experience fear conditioning paradigms. In fear conditioning study, a rat may be exposed to an electric shock while also being presented with a light. In subsequent trials the rat will display a fear reaction to the light alone because it has developed a conditioned fear response in which it associates the light with an aversive consequence. When the rat is subsequently presented with trials in which the light is presented without the electric shock, it learns that the light is harmless, and it achieves new (extinction) learning that inhibits the initial conditioned fear response.

The natural adaptation after trauma can be understood in a similar way. There is much evidence that the majority of people will display marked stress reactions in the initial weeks after trauma but that most of these reactions subside in the following few months. For example, whereas 94% of rape victims display sufficient PTSD symptoms 2 weeks posttrauma to meet criteria (excluding the 1-month time requirement), this rate drops to 47% 11 weeks later (Rothbaum et al. 1992). Similarly, rates of PTSD reduce by more than 50% following nonsexual assault (Riggs et al. 1995) and MVAs (Blanchard et al. 1996a). Even responses following the terrorist attacks of 11 September 2001 suggest that the normative response is to be resilient follow-

ing an initial period of distress. Five to 8 weeks after the attacks, 7.5% of a random sample of adults living south of 110th Street in Manhattan had developed PTSD, and of those living south of Canal Street, 20% had PTSD (Galea et al. 2002). In February 2002, another study found that in adults living south of 110th Street, only 1.7% of the sample had PTSD related to the attacks (Galea et al. 2005).

This pattern suggests that exposure to a traumatic event (unconditioned stimulus) leads to a strong fear reaction (unconditioned response), which is experienced by most trauma survivors. In the following weeks and months, however, most people learn that the many stimuli that were associated with trauma are no longer dangerous, and accordingly their fear reactions undergo extinction. In contrast, a minority of people develop strong conditioned responses such that when they are exposed to reminders of the trauma (conditioned stimuli), they experience strong fear reactions (conditioned response) (Charney et al. 1993; Fairbank and Keane 1982; Keane et al. 1985). For example, a man traumatized by a tsunami may have strong fear reactions and reexperiencing symptoms each time he is exposed to waves, or even the sound of running water, because these stimuli have been strongly conditioned to the fear reaction that occurred when he was threatened by the tsunami. It has been hypothesized that extreme sympathetic arousal at the time of a traumatic event may result in the release of stress neurochemicals that stimulate norepinephrine release in the amygdala, mediating an overconsolidation of trauma memories (Pitman et al. 2000; Cahill and McGaugh 1996). The reexperiencing symptoms of spontaneous intrusive thoughts, nightmares, and flashbacks have been posited to reflect this overconsolidation of trauma memories (Pitman et al. 2000). These reexperiencing symptoms may trigger the release of additional stress hormones, which further strengthen the trauma memories. In this way, PTSD responses can be regarded as failed extinction learning after the initial fear conditioning that occurs at the time of the trauma (Charney et al. 1993; LeDoux and Gorman 2001).

There is convergent evidence in support of the conditioning model. Several studies have demonstrated that combat-relevant audiovisual cues evoke larger increases in heart rate (HR) in Vietnam veterans with PTSD compared with nonveterans (Blanchard et al. 1982) and veterans without PTSD (Pallmeyer et al. 1986; Blanchard et al. 1986). This heightened reactivity appears to be specific to trauma-related stimuli. Veterans with PTSD have shown larger HR responses and increased frequency of skin resistance responses to combat stimuli but not to neutral stimuli, compared with noncombat psychiatric control participants (Malloy et al. 1983). Larger skin conductance (SC) responses to verbal presentations of trauma-related cues have also been demonstrated in Vietnam veterans with PTSD, compared with their responses to neutral words, and with the responses of veterans with other psychiatric disorders (McNally et al. 1987). Studies have also explored the responses of individuals with PTSD using their own recollections of the trauma as the eliciting stimulus. When individuals with PTSD have employed internal script-driven imagery to recall past traumatic events, they have consistently produced larger psychophysiological responses, including HR, SC, and facial electromyogram (EMG), compared with individuals with a similar trauma history without PTSD. This has been demonstrated across various trauma populations, including Vietnam veterans

(Pitman et al. 1987), Korea and World War II veterans (Orr et al. 1993), female victims of childhood sexual abuse (Orr et al. 1998), victims of terrorist attacks and other civilian traumas (Shalev et al. 1997, 1993), and breast cancer patients (Pitman et al. 2001). Similar findings have been reported in MVA victims with PTSD compared with MVA victims without PTSD when exposed to idiosyncratic audiotaped descriptions of their accident (Blanchard et al. 1996b). Notably, elevated psychophysiological reactivity to trauma-related imagery does not appear to be the result of having an anxiety disorder per se, because psychophysiological reactivity was not heightened in combat veterans with a non-PTSD anxiety disorder (Pitman et al. 1990). The results of these studies are consistent with the fear conditioning model of PTSD. Evidence that limbic networks are involved in the control of SC, HR, and blood pressure (Boucsein 1992; Davis 2000) support the interpretation that these responses may reflect the activation of fear conditioning networks (Charney et al. 1993; LeDoux 1996).

There is also evidence that elevated sympathetic activation in the acute trauma phase is associated with the subsequent development of PTSD. Several prospective studies assessed resting HR in trauma victims (Shalev et al. 1998; Zatzick et al. 2005) and MVA victims (Bryant et al. 2000) in hospital settings. Elevated tonic HR in the week after trauma was found in those who subsequently developed PTSD compared with those who did not develop PTSD (Shalev et al. 1998; Bryant et al. 2000; Zatzick et al. 2005). These data are consistent with the proposal that increased adrenergic activity may facilitate the consolidation of trauma memories underlying PTSD development (Pitman et al. 2000). It should be noted, however, that one study failed to replicate these findings (Blanchard et al. 2002).

There is also evidence that levels of posttraumatic stress after trauma exposure are predicted by the extent to which individuals are prone to conditioning prior to trauma exposure. In a study of firefighters before and after trauma exposure, one study reported that levels of acoustic startle response before trauma (reflecting the level of unconditioned response) predicted subsequent acute stress reactions (Guthrie and Bryant 2005a). It was also found that impaired extinction learning prior to trauma exposure significantly predicted subsequent posttraumatic stress (Guthrie and Bryant 2005b).

In summary, there is convergent evidence that development of PTSD can be compared with fear conditioning models, and that successful adaptation to a traumatic experience, including the successful resolution of symptoms through CBT, can be understood as a form of extinction learning (Davis and Myers 2002). Although this construct cannot explain all aspects of PTSD resolution, it is a useful framework to expand out understanding of treatment. It can be especially helpful in bridging the knowledge between neuroscience and CBT.

6. How Can Neuroscience Inform Cognitive Behavior Therapy?

On the premise that fear reduction is a learnt response, commentators have pro-

posed that extinction learning may be enhanced by modulating neurotransmitters that mediate learning. There is considerable evidence that glutamate plays an important role in learning and memory (Walker et al. 2002). Extending this notion to the study of fear, earlier evidence indicated that infusions of NMDA receptor antagonists impaired fear conditioning (Miserendino et al. 1990). Researchers subsequently began investigating the possibility that modulating NMDA receptors in the amygdala may also influence extinction learning (Falls et al. 1992). Subsequent findings indicated that NMDA receptors in the amygdala were important in fear extinction (Davis and Myers 2002).

In more recent years, attention has focused on the possibility of enhancing extinction learning by employing NMDA agonists. Specifically, D-cycloserine (DCS), which is a partial agonist at the NMDA glutamatergic receptor, has been shown to facilitate learning in animal (Flood et al. 1992) and human (Monahan et al. 1989) studies. DCS is particularly useful in the context of human learning because it is used in the treatment of tuberculosis, and clinically effective doses are not associated with aversive side effects. The utility of DCS has been indicated by repeated demonstrations that administration of DCS prior to extinction learning in rats facilitates reduction of the fear response (Ledgerwood et al. 2003; Walker et al. 2002). The possibility of enhancing the gains of CBT by modulating glutamatergic activity is indicated by recent pilot evidence in height phobics (Ressler et al. 2004). In this study, height phobic patients were administered exposure therapy via a virtual reality paradigm that involved incremental increases in height (Ressler et al. 2004). Prior to their two sessions of exposure therapy, these patients received either 50 mg of DCS, 500 mg of DCS, or placebo. There was no differential outcome between the two dosages, but both dosages led to greater reductions in anxiety during exposure therapy than the placebo. This finding suggests that treating posttraumatic stress may be facilitated by modulating glutamatergic activity through DCS prior to exposure therapy.

Although this proposal is yet to be tested in the context of PTSD, this avenue of research points to new directions in which neuroscience can inform CBT. By recognizing the similarities between the treatment of human response to trauma and extinction learning in animal fear conditioning paradigms, it is possible to extend theoretical models of PTSD, as well as to improve clinical procedures.

7. How Can Cognitive Behavior Therapy Inform Neuroscience?

The notion that CBT is a form of extinction learning also points to opportunities for greater understanding of the neural patterns associated with extinction learning after strong fear conditioning. Through successful therapy of PTSD we can identify neural networks that are associated with symptom reduction, and this in turn can elucidate how new learning inhibits prior fear conditioning.

Preclinical research has elucidated the neural underpinnings of fear conditioning (LeDoux 1995, 1998; Davis 1992). Brain structures activated during aversive con-

ditioning procedures are highly preserved across species; thus, inferences about neural structures implicated in human fear and anxiety can be extrapolated from preclinical studies (LeDoux 1996). The amygdala has been implicated in the processing of the emotional significance of an environmental stimulus (LeDoux 1998), and there is now converging evidence from a variety of experimental techniques demonstrating a crucial role of the amygdala in the acquisition and expression of conditioned fear (Davis 2000). Specifically, peripheral sensory signals are projected to the lateral nucleus of the amygdala (LA) via convergent thalamic and sensory cortical pathways (LeDoux et al. 1990, 1991; Li et al. 1996). LeDoux (1995) has proposed that the amygdala may be activated by rapid but rudimentary thalamic sensory inputs suggestive of threat to the organism, and it may be subsequently inhibited if slower, more detailed cortical inputs indicate the stimulus is harmless. LA sends projections to the central nucleus (CE) of the amygdala, which controls the behavioral, autonomic, and endocrine responses to aversive environmental stimuli via efferent projections to brainstem, limbic, and hypothalamic nuclei (LeDoux 1995). Accordingly, biological models of PTSD have hypothesized amygdala-mediated fear conditioning to underlie the disorder (Charney et al. 1993; Kolb 1987).

There is converging evidence that extinction is a form of new learning, rather than “unlearning,” which inhibits the expression of the conditioned response (Bouton 1988, 2000). It has been proposed that extinction involves the formation of a second, context-specific, inhibitory association between the CS and UCS representations (Davis and Myers 2002). The inhibitory association is posited to exist alongside the excitatory association, and counter the propensity of the excitatory association to activate the UCS representation (Davis and Myers 2002). There is some support for a role of the cortex in extinction. In a recent study, brief electrical stimulation of the medial prefrontal cortex paired with a conditioned stimulus facilitated extinction in rats (Milad and Quirk 2002). Lesions of the medial prefrontal cortex have also been found to retard the extinction of CRs in rats (Morgan et al. 1993), although a later study did not replicate this finding (Gewirtz et al. 1997).

In terms of PTSD, some models extend this evidence from animal studies to propose that reduced mPFC activity leads to impaired inhibition over amygdala fear processing networks, resulting in amygdala hyperresponsivity in PTSD (Bremner et al. 1999). Reduced activation in the medial prefrontal regions in PTSD patients has been reported in a number of brain imaging studies (e.g., Liberzon et al. 1999; Shin et al. 2004), but not all (e.g., Gilboa et al. 2004). However, this reduced cortical activity has not always been associated with increased amygdala activation (e.g., Bremner et al. 1999; Shin et al. 1999; Lanius et al., 2001).

According to this model, it would be hypothesized that successful response to CBT would lead to increased involvement of the mPFC as individuals engage in extinction learning and rehearse techniques to inhibit their anxiety. Preliminary evidence for this proposal comes from recent research that presented fearful and neutral faces to patients with PTSD and control participants and found marked reductions in mPFC activity in PTSD participants when processing the fearful faces (Williams et al. 2005). After these PTSD participants were treated with CBT, however, there were significant increases in mPFC activity (Felmingham et al. manuscript in

preparation). Although preliminary, these pilot data underscore the opportunities for CBT to shed light on the neural networks associated with successful new learning that inhibits initial fear conditioning.

8. Conclusion

By applying the construct of extinction learning to treatment of PTSD, there is much opportunity for greater interaction between CBT and neuroscience. It is important to recognize that extinction has limited explanatory application for PTSD, and the heterogeneous nature of PTSD precludes the reliance on extinction learning as an adequate construct to explain all features of adaptation after trauma. This qualification notwithstanding, the capacity to directly compare the acquisition and reduction of fear responses after trauma with parallel phenomena in preclinical animal and human studies of the fear response will allow us to extend our conceptual understanding of the neural mechanisms underpinning treatments of PTSD. The clinical opportunities that emerge as a result of this extension of CBT are demonstrated by the study of using DCS to facilitate treatment outcome. It is most likely that CBT will be further extended as lessons from neuroscience are applied to psychological means of helping those affected by traumatic experiences.

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Pharmacological Interventions for Posttraumatic Stress Disorder

Murray B. Stein

1. Introduction

The purpose of this chapter is to review the current evidence base for treatment of posttraumatic stress disorder (PTSD) with pharmacological agents. For the most part, the scope of this review is limited to randomized controlled trials (RCTs) of pharmacotherapies, although where such data are sparse or nonexistent for specific agents, other evidence (e.g., open-label trials) will be included. Pharmacotherapy whose goal is to prevent the occurrence of PTSD shortly after trauma exposure is covered in another chapter in this volume (see the chapter by Pitman, this volume). Cognitive and behavioral treatments are also covered in another chapter in this volume (see the chapter by Bryant, this volume). However, brief commentary on the use of combined (i.e., pharmacological and psychological) treatments is provided here.

2. Antidepressants

2.1. Tricyclic Antidepressants and Monoamine Oxidase Inhibitors

In a double-blind RCT, the efficacy of the tricyclic antidepressant (TCA) imipramine and the monoamine oxidase inhibitor (MAOI) phenelzine were compared with that of placebo in 60 male veterans with PTSD (Kosten et al. 1991). Both active medications proved superior to placebo in reducing PTSD symptoms, with some measures favoring phenelzine over imipramine. Although phenelzine was promising in this study, there are, unfortunately, no other published RCTs with MAOIs for PTSD. Despite the difficulties with diet and other adverse events that limit the use of MAOIs, the often treatment-refractory nature of PTSD makes it all the more important that studies be conducted with this class of medication to confirm its poten-

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tial utility, particularly for patients who fail to respond to selective serotonin reuptake inhibitors (SSRIs).

In a small, double-blind, crossover study, 18 male combat veterans with PTSD completed 4 weeks of treatment with 200 mg/day of desipramine compared with placebo (Reist et al. 1989). No improvement for desipramine over placebo was detected in PTSD-specific symptoms although there was improvement, as would be expected, in depressive symptoms. This study may have been too small, and almost certainly too short in duration, to detect the potential benefits of desipramine for PTSD symptoms.

Davidson et al. (1990) conducted an 8-week RCT of amitriptyline in 46 veterans with chronic PTSD. Amitriptyline was superior to placebo on several measures of PTSD symptoms, including overall recovery rates. In this study, at the end of treatment, 64% of amitriptyline-treated and 72% of placebo-treated patients still met diagnostic criteria for PTSD. This is one of the first studies to note that even among those patients who “improve” with antidepressant treatment, many continue to experience residual symptoms.

There is very limited evidence for the possible utility of a tricyclic antidepressant, imipramine, to treat symptoms of acute stress disorder (ASD). In an RCT of pediatric burn patients, 25 children (aged 2–19 years) received either imipramine (1 mg/kg, maximum 100 mg, at bedtime) or chloral hydrate (25 mg/kg, maximum 500 mg, at bedtime) for 7 days (Robert et al. 1999). Imipramine was more effective than chloral hydrate in treating ASD symptoms: only 5 of 13 (38%) children responded to chloral hydrate, compared with 10 of 12 (83%) children who responded to low-dose imipramine. More studies will be needed, looking at both safety and efficacy, before tricyclic antidepressants can be considered for use in this context.

2.2. *Selective Serotonin Reuptake Inhibitors*

Selective serotonin reuptake inhibitors were first approved for the treatment of depression, but they have subsequently been shown to be efficacious for the treatment of a number of anxiety disorders, including PTSD. SSRIs are known to enhance serotonin functioning in the central nervous system. However, the mechanism by which they effect improvement in mood and anxiety disorders is uncertain. It possibly involves effects on second-messenger systems and/or neuropeptides such as brain-derived neurotrophic factor (BDNF) (Duman et al. 2001; Malberg and Duman 2003).

2.2.1. Acute Treatment with SSRIs

van der Kolk and colleagues were among the first to compare the effectiveness of fluoxetine with placebo in civilians with PTSD ($n = 23$), and in Vietnam combat veterans with PTSD ($n = 24$) (van der Kolk et al. 1994). The percentage reduction in symptom severity was 40% for the civilians and 15% for the veterans, leading to the oft-stated contention that combat-related PTSD may be less responsive to phar-

macotherapy. Given the large numbers of veterans of new wars (e.g., in Iraq and Afghanistan, in the case of US troops) now returning with PTSD (Kang and Hyams 2005), it will be very important to determine whether new-onset PTSD in this context is responsive to SSRIs.

Larger controlled studies followed soon after these initial reports of SSRI efficacy, leading to the regulatory approval in many countries of certain members of this class of drugs as the first form of pharmacotherapy sanctioned for PTSD. Several large, multicenter trials served as the primary basis of approval for two SSRIs, sertraline and paroxetine, by the United States Food and Drug Administration (FDA) (Brady et al. 2000; Davidson et al. 2001b). Sertraline administered for 12 weeks was moderately more effective (effect sizes ≈ 0.5) than placebo in 187 individuals (Brady et al. 2000) and in 208 individuals (Davidson et al. 2001b) with PTSD. Paroxetine administered for 12 weeks was also moderately more effective (effect sizes ≈ 0.5) than placebo in two trials, one with 551 individuals with chronic PTSD (Marshall et al. 2001), and another with 307 individuals with PTSD (Tucker et al. 2001).

Although not FDA-approved for PTSD, fluoxetine also has shown benefit compared with placebo in two 12-week RCTs. In an RCT of 53 civilians with PTSD, fluoxetine administered for 12 weeks showed a marked (effect size ≈ 1.0) improvement over placebo (Connor et al. 1999b). In a study of 301 persons with PTSD, of whom 81% were men and 48% were combat veterans, fluoxetine showed a moderate (effect size ≈ 0.4) improvement over placebo (Martenyi et al. 2002).

Given the evidence that several members of the SSRI class are efficacious for treating PTSD, there is every reason to believe that this is an effect shared by the class, and that any of the SSRIs could be used for this purpose. Thus, choice among the SSRIs for a given patient would involve consideration of factors such as side effects, prior response, and perhaps family history of response. Dosages of SSRIs for the treatment of PTSD are in the same range as those recommended for major depressive disorder (MDD). Because many patients with PTSD suffer from comorbid MDD (Shalev et al. 1998), the use of SSRIs is expected to benefit both conditions.

2.2.2. Duration of Treatment with SSRIs

There are several studies that confirm the notion that longer treatment with SSRIs is better. Lønborg et al. (2001) continued 128 patients with PTSD who had completed 12 weeks of double-blind, placebo-controlled, acute-phase treatment with sertraline into a 24-week open-label continuation phase. In addition to noting that 92% of responders to the initial 12 weeks of treatment maintained their response during the full 6 months of continuation treatment, investigators found that 54% of nonresponders to the initial 12 weeks of treatments converted to responder status during continuation therapy. Furthermore, they noted that patients with higher pretreatment PTSD severity required longer (i.e., more than the initial 12 weeks) to respond.

Davidson et al. (2001a) examined the efficacy of sertraline, compared with placebo, in sustaining improvement and preventing relapse over 28 weeks in patients

with PTSD. Ninety-six patients who completed the aforementioned study (Londborg et al. 2001) were enrolled in a RCT of 28 weeks of maintenance treatment with sertraline (50–200 mg, $n = 46$; 78% women) or placebo ($n = 50$; 62% women). Continued treatment with sertraline yielded lower PTSD relapse rates (5%) than placebo (26%). Patients who received placebo were 6.4 times as likely to relapse as were patients who received sertraline.

Taken together, results of these two studies provide evidence for the merits of patiently waiting long enough (i.e., beyond 3 or 4 months, which may only be clinically feasible if there is at least some initial response) for the full benefits of SSRIs to accrue, and for the ability of continued treatment to provide protection against relapse. The current recommendation from a panel of experts is that PTSD pharmacotherapy with SSRIs should continue for a minimum of 12–24 months (Stein et al. 2003). Of considerable interest will be studies that look at combining cognitive behavioral therapies with pharmacotherapy, with the aim of providing longer, more durable effects, if and when medication is discontinued.

2.3. Other Antidepressants

2.3.1. Dual Reuptake Inhibitors

Clinical experience with dual reuptake inhibitors (e.g., venlafaxine extended-release or duloxetine) suggest that these may be useful (Gorman and Kent 2002), although no RCTs have yet been published with these agents in PTSD.

2.3.2. Nefazadone

Nefazadone is an antidepressant with antagonism at the 5-HT₂ receptor as well as inhibition of serotonin reuptake and antagonist activity at the alpha₁-adrenergic receptor. It may be especially useful in treating sleep problems associated with PTSD (Gillin et al. 2001), although published experience with this medication for PTSD is limited. Nefazadone has recently been withdrawn from the market in many countries as a result of concerns about its hepatotoxicity, which, in some cases, was associated with liver failure. Thus, its use is more of theoretical than practical interest at this point.

2.3.3. Mirtazapine

Mirtazapine has effects that involve enhancement of both noradrenergic and serotonergic function. An open-label trial with mirtazapine demonstrated significant reduction in PTSD symptoms in 6 patients with severe PTSD (Connor et al. 1999a). A subsequent small, double-blind, placebo-controlled RCT in 29 patients with PTSD found superiority of mirtazapine (up to 45 mg/day) on most measures of PTSD severity, leading the authors to conclude that larger, more definitive studies should be conducted (Davidson et al. 2003). So far, no such studies have appeared in the literature.

2.3.4. Bupropion

Although the mechanism of action of bupropion is unclear, there is some evidence that it has dopaminergic and noradrenergic agonist activities. An open-label study of 17 veterans with PTSD did not demonstrate significant improvement of PTSD symptoms after 6 weeks (Cañive et al. 1998).

3. Anticonvulsants

Numerous anticonvulsants, including carbamazepine, valproic acid, lamotrigine, gabapentin, topiramate, and tiagabine, have been used to treat a variety of psychiatric disorders. Among the many neuropsychiatric indications for which anticonvulsants have been proposed as potentially useful, PTSD has been a focus with some preliminary, mixed results.

Two open-label studies in combat veterans with PTSD, one with carbamazepine ($n = 10$) (Lipper 1989), and one with valproic acid ($n = 16$) (Fesler 1991), found significant improvement in approximately two thirds of patients.

The benefits of lamotrigine were investigated in a 12-week double-blind, placebo-controlled RCT (Hertzberg et al. 1999). This study randomized 25 patients in a 2:1 ratio to either lamotrigine or placebo. The response rate in the lamotrigine group was 50% compared to 25% in the placebo group. Larger RCTs are required to confirm the promising results of these pilot data.

Tiagabine, a selective GABA reuptake inhibitor, was evaluated as an add-on therapy in an open-label study of seven consecutive female patients with PTSD (Taylor 2003). Tiagabine was started at 2 mg nightly and increased by 2-mg increments every 2 to 3 days until an optimal response was achieved. Tiagabine (mean dose 8 mg/day, range 4–12 mg/day) markedly improved PTSD symptoms within 2 weeks in six of the seven patients. These preliminary, promising findings warrant further study into the efficacy and safety of tiagabine for the treatment of PTSD.

Berlant and van Kammen (2002) reported results of a retrospective chart review of 35 patients with chronic civilian PTSD who were administered topiramate, 12.5 mg/day to 500 mg/day as add-on or monotherapy for treatment-resistant PTSD. Berlant (2004) also conducted a prospective, open-label trial of topiramate as add-on or monotherapy in 33 patients with civilian PTSD. The impression from both studies was that topiramate was helpful for some patients, and that prospective RCTs should be conducted.

4. Benzodiazepines and Other Hypnotics

Although sleep problems are common and notoriously difficult to treat in PTSD

(Lavie 2001), and benzodiazepines and other hypnotics are widely used as adjunctive treatment in PTSD (Mellman et al. 2003), it is surprising that there are very few controlled studies of the use of benzodiazepines or other hypnotics for PTSD. The largest of these included 16 patients with chronic PTSD who participated in a 12-week double-blind, crossover study of alprazolam and placebo (5 weeks with each, and an intervening 2-week washout period) (Braun et al. 1990). In this small study, there was evidence of benefits of alprazolam over placebo on the core symptoms of PTSD. Given the extent to which benzodiazepines are used in PTSD, with virtually no evidence supporting their efficacy, the need for definitive RCTs is apparent and pressing.

5. Adrenergic Agents

Several small, open-label studies with the alpha-1-adrenergic agonist clonidine have suggested that it may be useful in combination with antidepressants to augment treatment in PTSD, with a particular focus on reduction of hyperarousal symptoms (Harmon and Riggs 1996; Kinzie and Leung 1989). However, in the only published RCT with an alpha-1-adrenergic agent (in this case guanfacine, which is associated with fewer adverse effects, such as sedation, than clonidine), there was no evidence of significant improvement in 29 guanfacine-treated patients compared with 34 placebo-treated patients with chronic PTSD (Horrigan and Barnhill 1996).

In contrast to these negative findings with guanfacine, an alpha-1-receptor *agonist*, prazosin, was shown to be efficacious in a 20-week, double-blind, placebo-controlled crossover RCT of 10 male Vietnam combat veterans (Raskind et al. 2003). Prazosin was well tolerated, even at doses that averaged 9.5 mg/day (given at bedtime). Sleep symptoms such as nightmares were especially improved with prazosin. It is hoped that larger RCTs will be conducted to confirm these promising results.

6. Antipsychotics

As recently reviewed, numerous trials have demonstrated the usefulness of adjunctive antipsychotic medications for treating psychotic symptoms in patients with PTSD (Hamner et al. 2004). As also noted in that review, there is additional evidence that adjunctive antipsychotics may be useful in the treatment of nonpsychotic patients with refractory PTSD symptoms. Two RCTs have been conducted with the focus on treatment resistance in nonpsychotic patients with PTSD. In the first of these, Stein et al. (2002) administered adjunctive olanzapine (escalated up to 20 mg/day, as tolerated) or placebo to 19 male combat veterans with SSRI-resistant PTSD. In that study, olanzapine coadministration was associated with statistically significant and clinically relevant benefits compared with placebo on PTSD symp-

toms, specifically sleep disturbance; however, weight gain associated with olanzapine was a concern.

Bartzokis et al. (2005) evaluated the efficacy and safety of risperidone in 73 veterans with chronic combat-related PTSD who were referred to a residential treatment program. Risperidone was added to a stable psychotropic medication regimen in 92% of subjects. Of 48 patients who completed the 4-month study (which began on an inpatient basis and was continued on an outpatient basis), significantly greater improvement in PTSD symptoms was obtained in subjects who received risperidone compared with placebo. Taken together, these studies support the notion that atypical antipsychotic medications may be useful as adjunctive treatment in PTSD, and they call for larger RCTs to confirm these preliminary observations.

7. Conclusion

As recently noted in a review of PTSD diagnosis and treatment (Stein 2002), the evidence base for PTSD treatment is a work in progress. There is a pressing need to develop medication treatments that are generally more effective, and better tolerated (which would facilitate compliance in the longer term). In particular, the need to develop pharmacological treatments that better target sleep problems is apparent. An important unanswered question, given the efficacy of cognitive behavioral therapies (CBTs) for PTSD (see the chapter by Bryant, this volume), is when pharmacotherapy is indicated vis a vis psychotherapy and, especially, whether the combination is likely to be most effective of all. From the few RCT studies for which acute effect sizes can be estimated, it would appear that CBTs have an edge over pharmacotherapy (Stein 2002). Furthermore, whereas pharmacotherapy continues to work as long as the medication is taken, the effects of CBT persist beyond the actual treatment itself (seen up to 6 and 12 months, respectively, in two RCTs) (Foa et al. 1999; Marks et al. 1998). It must be emphasized, however, that the putative advantages of one treatment modality over the other are at present speculative, as no published study has yet to compare acute or long term outcomes of pharmacotherapy to any psychosocial treatment, or to the combination thereof. These are areas where additional research is sorely needed.

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Secondary Pharmacological Prevention of PTSD: Therapeutic Implications of a Translational Model

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1. Introduction

“Significance facilitates remembrance” (McGaugh 1990). Millions of Asians are likely to remember in vivid detail where they were and what they were doing on the morning of 26 December 2004, when the disastrous tsunami struck the shores of the Indian Ocean. In contrast, few of these persons are likely to remember in any detail where they were and what they were doing on the morning of 19 December, only a week earlier. We are more likely to remember significant life events than trivial ones, and this is surely the result of natural selection. Suppose a hypothetical primitive hominid decided to take a new route to a watering hole, and on her way she encountered a crocodile. Should she fail to remember in the future that a crocodile inhabited that route, she would be more likely to take the same route again and be eliminated from the gene pool!

Evolution appears to have enabled significance to facilitate remembrance by means of modulatory effects exerted by neurohormones on the consolidation of memory traces, or alternately stated, on the acquisition of conditioned emotional responses. Because emotionally arousing events mobilize neurohormones, facilitation of learning by these hormones amounts to a mechanism whereby the intensity of the unconditioned emotional response to an arousing event regulates the strength of the resultant conditioned response. Evolution favors parsimony; if it can achieve two adaptations through one mechanism, it often will. In the above hypothetical example, the same adrenaline (or epinephrine, EPI) that enabled our primitive hominid to run away from the crocodile acted in her brain to strengthen her memory of the frightful encounter.

2. The Role of Stress Hormones

Stress hormones that have been shown to facilitate memory and conditioning in ex-

perimental animals include not only EPI, but also corticotropin releasing hormone, adrenocorticotropin (ACTH), arginine vasopressin (AVP), and cortisol (Croiset et al. 2000; McGaugh and Roozendaal 2002). Exogenous administration or endogenous activation of these substances shortly following a learning trial leads to the formation of a conditioned response (CR) that is stronger and more resistant to extinction. Their pharmacological blockade produces the opposite effect. This body of findings represents one of the most exciting discoveries in the history of behavioral neuroscience (McGaugh 2003).

In rats trained in a passive avoidance task, retention is enhanced by systemic posttraining injections of EPI (Gold and van Buskirk 1975). This finding has been replicated in numerous independent experiments. The memory-enhancing effect of EPI is counteracted by the pretraining administration of the β -adrenergic receptor blocker propranolol (Sternberg et al. 1985). Posttraining administration of systemic propranolol to rats also impairs subsequent memory for a stressful spatial water maze task (Cahill et al. 2000). One creative study found that oral propranolol abolishes the memory-enhancing effect of negative emotional arousal in humans (Cahill et al. 1994). Although propranolol interferes with sympathetic β -adrenergic transmission both peripherally and centrally, evidence suggests that its central action is responsible for blocking memory enhancement. The β -adrenergic blocker nadolol, which does not cross the blood-brain barrier, does not share this effect of systemic propranolol (van Stegeren et al. 1998).

3. The Role of the Amygdala

The basolateral nucleus of the amygdala (BLA) appears to be the critical brain structure involved in both fear conditioning (Davis 1990; LeDoux 1996) and the memory-enhancing effects of emotional arousal (McIntyre et al. 2003). Posttraining intra-BLA microinjections of norepinephrine (NE) enhance conditioning, and this effect is blocked by simultaneous intra-BLA administration of propranolol (Liang et al. 1986). β -Adrenergic neurotransmission in the BLA is a final common pathway for the influence of most stress hormones on memory, and propranolol acts to block this pathway (McGaugh et al. 2002).

4. A Translational Model of PTSD Pathogenesis

Pitman (1989) advanced a novel theory of the pathogenesis of posttraumatic stress disorder (PTSD) based upon the above animal research into the memory-enhancing effects of stress hormones. Specifically, he postulated that in trauma victims who go on to develop PTSD, the traumatic event (unconditioned stimulus, UCS) stimulates an excessive release of stress hormones (UCR), which over-consolidate memories of the event (*Consolidation* loop in Fig. 1), which subsequently manifest themselves

in the intrusive recollections and reexperiencing symptoms found in PTSD. Pitman further hypothesized that reminders of the traumatic event (conditioned stimuli, CS) lead to retrieval of the traumatic memories, with the additional release of stress hormones (CR). These further enhance the strength of the traumatic memory, thereby creating a positive feedback cycle (*Paradoxical Enhancement/Reconsolidation* loop in Fig. 1). This model regards PTSD as a quantitative overshoot of a normally adaptive mechanism, in which too much significance leads to too much remembrance.

There are several lines of evidence in humans that support the hypothesized pathogenesis of PTSD depicted in the *Consolidation* loop in Fig. 1. DSM-IV diagnostic criterion A.2 for PTSD requires an acute response to the traumatic event of "intense fear, helplessness, or horror," which is highly likely to mobilize stress hormones such as epinephrine. Elevated heart rate (HR) in the aftermath of a traumatic event, indicative of a hyperadrenergic state, has been found to predict subsequent PTSD in six (Bryant et al. 2000; Bryant et al. 2004; Kassam-Adams et al. 2005; Nugent et al. 2005; Shalev et al. 1998; Zatzick et al. 2005) out of eight (Blanchard et al. 2002; Buckley et al. 2004) studies. A recent study of children found that post-

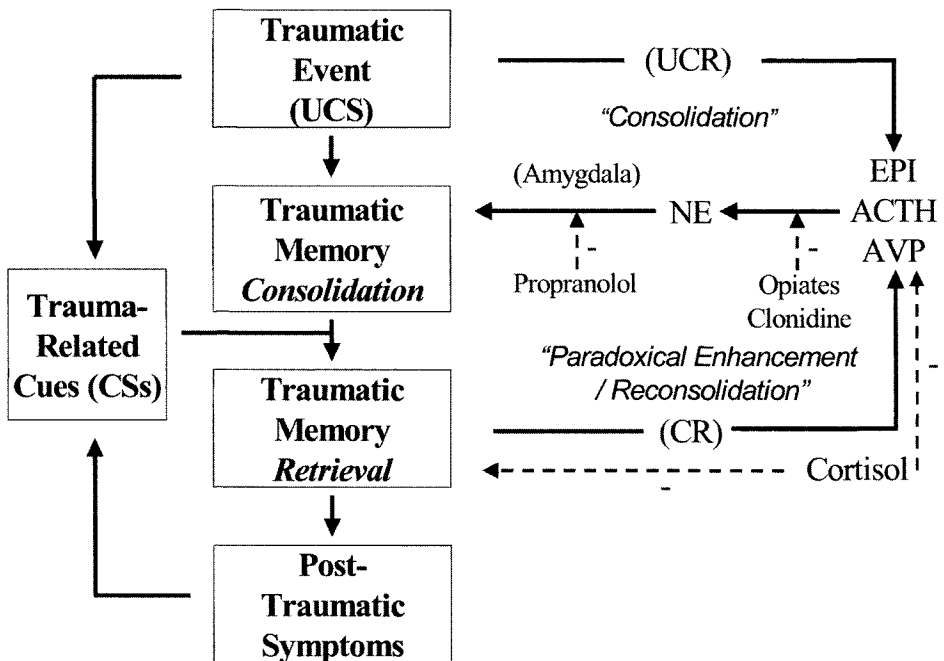


Fig. 1. Hypothetical model of posttraumatic stress disorder pathogenesis with points of opportunity for potential secondary and tertiary preventive psychopharmacologic interventions. UCS, unconditioned stimulus; UCR, unconditioned response; CS, conditioned stimulus; CR, conditioned response; EPI, epinephrine; ACTH, adrenocorticotropin; AVP, arginine vasopressin; NE, norepinephrine

trauma urinary EPI excretion positively predicted subsequent PTSD symptoms (Delahanty et al. 2005). The counterintuitive finding that quadriplegic injury is less often followed by PTSD than paraplegic injury (Radnitz et al. 1998) is potentially explained by the severing of the connection between the brain and the adrenal glands in the former but not in the latter condition. However, challenges to the pathogenic model of PTSD presented here are posed by the viewpoints that PTSD may not be properly regarded as a maladaptive process that occurs immediately following a trauma, but rather as a process involving the lack of resolution of an acute stress reaction; also that PTSD may not involve a normative response to an extreme trauma event, but rather an endocrinologically atypical response (McFarlane and Yehuda 2000).

The hypothetical capacity of conditioned responses to reinforce (rather than extinguish) conditioned responding has been termed "paradoxical enhancement" or "incubation." It has only been experimentally demonstrated in animal (Napolkov 1963) and human experiments (Campbell et al. 1964) that have employed exceptionally strong UCSs, which today would be regarded as unethical. However, strong UCSs characterize PTSD. Eysenck (1967, 1968) postulated that incubation plays a pathogenic role in human neurosis. Subsequently, Eysenck and Kelley (1987) hypothesized that neurohormones mediate incubation. Paradoxical enhancement has a possible neurophysiologic basis in the decreased ratio of inhibitory (medial prefrontal) over excitatory (anterior limbic, amygdala) central nervous functions observed in PTSD (e.g., Shin et al. 2004). The concept of paradoxical enhancement offers an explanation for the hitherto unexplained, troublesome ability of psychological debriefing in the aftermath of trauma to exacerbate its psychopathological effect (see below).

5. The Challenge of PTSD Prevention

Little is available in the way of prophylaxis (i.e., prevention) for this potentially disabling mental disorder. "Primary prevention" of PTSD involves the prevention of traumatic events. "Secondary prevention" involves intervening in the aftermath of a traumatic event to forestall the development of PTSD. "Tertiary prevention" involves interventions designed to reduce symptomatology and disability after PTSD has developed. The current state of PTSD mental hygiene consists largely of tertiary prevention, which is too often of limited benefit. Until recently, the most popular secondary preventive intervention for PTSD was psychological debriefing. However, recent reviews have failed to confirm its efficacy and have even suggested it may have adverse long-term effects (Bledsoe 2003; Rose et al. 2003).

The collapse of the empirical basis for debriefing underscores the need to find other secondary preventions for PTSD. Treating acute stress disorder with cognitive behavior therapy (CBT) has been reported to have preventive value for subsequent PTSD (Bryant et al. 1998). Pharmacological secondary prevention of PTSD has received little research attention, but is a topic of increasing medical interest (Larkin

1999). Medical and surgical house officers carry emergency care manuals that include many preventive actions. Examples include prescribing anticoagulants to prevent pulmonary embolism from venous thrombophlebitis and antibiotics to prevent secondary infection in burn victims. In these manuals, it is difficult to find mention of preventive mental health interventions, including any aimed at acutely traumatized persons.

6. Implications of the Translational Model for the Prevention of PTSD

If the model of PTSD pathogenesis presented above is applicable, the possibility exists that the disorder can be prevented by pharmacologically intervening in the aftermath of a traumatic event to block potentiation of memory consolidation by stress hormones. Blocking consolidation would be distinct from, but potentially complemented by, later preventive cognitive behavior therapy, which is likely to act by facilitating extinction. However, memory consolidation is a time-dependent process. There is a limited period during which the memory trace is “labile” and subject to the modulating influences of stress hormones. This raises the question as to how soon after the occurrence of a traumatic event a pharmacological agent would need to be administered to lower the risk of PTSD. The finding that injections of EPI administered to rats at intervals of 30 min or longer following single-trial avoidance training are generally ineffective in strengthening memory (McGaugh 1990) would pessimistically suggest that pharmacological intervention might have to occur sooner after traumatic exposure than would be practical. However, vasopressin given 3 h posttraining has been found to produce a 45% increment in memory performance during subsequent testing (Kovacs and Telegdy 1982). The effect of vasopressin became insignificant after 6 h, suggesting a wider but still limited labile period, or window of opportunity, during which pharmacological agents may be successfully administered. A study that employed positron emission tomography in humans found that it takes 6 h to permanently store the memory of a newly learned skill in the brain (Shadmehr and Holcomb 1997). More recent studies in rats have indicated that there appear to be several stages of memory consolidation involving different brain regions that may potentially be affected by pharmacological intervention for a number of days posttraining. For example, reversible inactivation of BLA by tetrodotoxin injection 2 days after a single-session acquisition paradigm impairs conditioned freezing responses when measured 48 h postinjection. In the case of perirhinal cortex injection, the window of opportunity is a full 8 days (Sacchetti et al. 1999).

Importantly, rats are not humans, and they do not develop the clinical syndrome of PTSD. One likely difference between a single-trial avoidance paradigm in rats and a traumatic event in humans is that humans may replay the traumatic event in their consciousness for variable periods of time after its occurrence (McFarlane et

al. 2002). Indeed, this is one of the diagnostic features of acute stress disorder. Re-experiencing may produce sympathetic arousal (Pitman et al. 1987), and such recurrent arousal may further influence memory consolidation, as suggested in the *Paradoxical Enhancement/Reconsolidation* loop of Fig. 1. Thus, there may be continuing hormonal influences on memory that persist for longer periods (i.e., days or weeks) following a traumatic event, which may be amenable to pharmacological intervention. Ultimately, the question of the window of opportunity for influencing the development of PTSD can only be resolved by clinical trial.

7. Adrenergic Blockers

Based upon the animal research to date, the most promising candidate drug for intervening in the aftermath of a traumatic event to block potentiation of the consolidation of its memory trace by stress hormones is propranolol, which as noted above acts to block postsynaptic β -adrenergic receptors in the BLA. Support for the ability of posttrauma propranolol to attenuate the subsequent development of PTSD comes from two published but preliminary studies. Pitman et al. (2002) recruited 41 patients who presented to a general hospital emergency room (ER) immediately following a traumatic event (mostly motor vehicle accidents, or MVAs). To be eligible, patients had to meet the DSM-IV PTSD A.1. (event) and A.2. (response) criteria. Furthermore, patients had to have a pulse rate of 80 beats per minute (BPM) or higher, presumably indicative of a hyperadrenergic state. The patients were randomized to receive a course of 40 mg oral propranolol or placebo four times daily for 10 days, followed by a 9-day medication taper period. The taper period ended approximately 12 days prior to the first outcome assessment, so that patients were fully withdrawn from the propranolol at the time of assessment. The first dose of study medication was administered an average of 4 h after traumatic event occurrence. One month posttrauma, total scores on the Clinician-Administered PTSD Scale showed a trend to be lower in the 11 completers of the course of study medication who had received propranolol, compared with the 20 completers who had not. Based upon their physiological responses during script-driven mental imagery of the traumatic event that had brought them to the ER 3 months earlier, 0 of 8 propranolol, but 8 of 14 placebo, patients were physiologically classified as PTSD ($P = 0.04$), which is consistent with a reduced conditioned fear response.

In a second, controlled, nonblind, nonrandomized, preliminary study, Vaiva et al. (2003) recruited 19 patients with HR of 90 BPM or greater from two ERs in France 2–20 h following an MVA or physical assault. All were offered 30 mg propranolol three times daily for 7 days followed by a taper period of 8–12 days. The investigators compared the 11 patients who agreed to take the propranolol with 8 patients who refused propranolol but agreed to participate in the study. The two groups did not differ on demographics, exposure characteristics, physical injury severity, or peritraumatic emotional responses. At 2 months posttrauma, levels of PTSD symptoms were significantly lower in the patients treated with propranolol. Eighty-six

percent of the time a patient who took propranolol had a score below that of a patient who did not ($P = 0.04$). Both the American and French groups are currently launching randomized clinical trials in larger samples in an attempt to obtain definitive results.

Finally, as described in a published single case study, a 44-year-old woman had experienced several prior MVAs, each followed by months of PTSD symptoms despite treatment with multiple drugs. Following yet another accident, severe PTSD symptoms again emerged, but these were rapidly and markedly reduced by propranolol 60 mg twice daily started 48 h after the event (Taylor and Cahill 2002).

8. Other Antiadrenergic Agents

Another approach to blocking the potentiation of the consolidation of a traumatic memory would be to give pharmacological agents that act presynaptically to reduce NE release. Candidate drugs for this purpose include opioids, α -2-adrenergic agonists such as clonidine and guanfacine, and γ -amino butyric acid (GABA)-ergic agents. Saxe et al (2001) performed an observational study of the relationship between the amounts of morphine given to severely burned children and their PTSD symptoms. The children who received higher doses of morphine had a greater reduction in PTSD symptoms over a 6-month hospital stay ($r = 0.44$, $P < 0.05$). However, an alternate explanation of this finding is that the analgesic properties of morphine reduced the intensity of repeated traumatic incidents such as painful dressing changes. Randomized controlled trials of the ability of opioids to prevent PTSD would appear to be indicated but are likely to be more difficult to implement due to the clinical bar to withholding them when severe pain is involved, as well as their addictive potential. To date there have been no reported studies of the use of α -2-adrenergic agonists to prevent PTSD.

γ -Amino butyric acid (GABA) agents can also oppose NE action in the BLA. Some GABAergic drugs have been found to reduce the potentiation of memory by stress hormones (Introini-Collison et al. 1994). Benzodiazepines, however, are less effective in this regard when administered posttraining rather than pretraining. This limitation may account for the failure of Gelpin et al. (1996) to find preventive value for PTSD of clonazepam or alprazolam administered in the acute aftermath of trauma. Nevertheless, given the ready availability of the benzodiazepines, and their current widespread use in psychologically traumatized persons, it would seem that further trials are indicated, either to search for some evidence of their preventive efficacy, or to establish the lack thereof and thereby alter practice patterns. Trials of newer GABAergic agents, e.g., gabapentin or tiagabine, which lack some of the undesirable effects of benzodiazepines, are also indicated.

9. Cortisol

At first blush, the model of PTSD pathogenesis presented herein would suggest that cortisol, which has also been found in animal research to potentiate memory consolidation when given posttraining, would be counter-preventive. The situation is complicated, however, by the fact that endogenous hypercortisolemia (Mauri et al. 1993) and exogenous glucocorticoid administration (Newcomer et al. 1999) have been found to impair human memory retrieval. Kirschbaum et al. (1996) found impaired performance in declarative memory tasks following a 10-mg dose of hydrocortisone (cortisol).

The ability of cortisol to impair memory retrieval is indicated by an arrow in the bottom right of Fig. 1. There it will be seen that such an effect has the capability to interrupt the positive feedback of the *Paradoxical Enhancement/Reconsolidation* loop. In other words, if PTSD patients' recall of their traumatic events could be reduced by cortisol, the hypothesized rerelease of stress hormones that traumatic recall induces, and the resultant paradoxical enhancement of conditioned responding, might be reduced as well.

Cortisol could also work indirectly by containing an overshoot of EPI ACTH, and AVP. One function of cortisol is to shut down the adrenergic hormones of the "fight or flight" response, preventing their long-term elevation from damaging the body (Munck et al. 1984; Ursin and Olff 1993). Yehuda and Harvey (1997) and Yehuda et al (1998) have suggested that during traumatic stress, catecholamine increases are likely to be exaggerated in the presence of a diminished regulatory influence of accompanying cortisol increase. In other words, lower cortisol levels at the time of the trauma may lead to a failure to contain the sympathetic stress response and to consequent prolonged availability of NE in the brain (Pacak et al. 1995). This may then lead to altered consolidation of memory of the traumatic incident (Yehuda 2002). In animals, low levels of cortisol have been shown to increase the memory-enhancing effects of catecholamines (Bohus 1984), and high doses of glucocorticoids to decrease these effects (Borrell et al. 1983).

Two observational studies have found that lower posttrauma cortisol levels predict subsequent PTSD. MVA victims who subsequently were diagnosed with PTSD had lower plasma cortisol levels 30 min after their accident than victims who subsequently met criteria for major depression. Victims not meeting criteria for either diagnosis had intermediate cortisol levels (McFarlane et al. 1997). Delahanty et al. (2000) examined the relationship between initial urinary hormone excretion and subsequent PTSD symptoms in 99 MVA victims. In this study, MVA victims were catheterized upon arrival to the trauma unit, and urine was collected for the next 15 h. Victims who subsequently met acute PTSD diagnostic criteria 1 month after the accident had significantly lower cortisol excretion in the immediate aftermath of the accident than victims who did not meet diagnostic criteria. In addition, initial cortisol excretion was negatively correlated with subsequent symptoms of PTSD. However, in a recent study of traumatized children, the same investigators (Delahanty et al. 2005) reported the opposite finding, i.e., a positive correlation between

posttrauma cortisol excretion and subsequent PTSD symptoms.

Investigations by Schelling and colleagues have found that exogenously administered stress doses of cortisol reduce the development of subsequent PTSD in medical-surgical patients. An initial retrospective case-control analysis revealed that septic shock patients who received a 100-mg bolus of hydrocortisone followed by $0.18 \text{ mg kg}^{-1} \text{ h}^{-1}$ until shock reversal had a significantly lower subsequent incidence of PTSD than patients who received standard treatment for their septic shock (Schelling et al. 1999). These findings were replicated in a randomized, double-blind study (Schelling et al. 2001). During a sepsis episode, 11 patients were randomly assigned to receive placebo, and 9 were assigned the abovementioned dose of hydrocortisone for 6 days. Results revealed that only 1 of 9 from the hydrocortisone group but 7 of 11 from the placebo group ($P = 0.02$) met PTSD criteria assessed 31 months after discharge from the intensive care unit. However, interpretation of this finding is confounded by the observation that the placebo patients required higher amounts of NE for vasopressor support. Thus, the exogenous cortisol could have acted indirectly to prevent PTSD by reducing total exogenous NE requirement.

More recently, Schelling et al. (2004) examined the efficacy of perioperative and postoperative exogenous hydrocortisone in preventing PTSD symptoms in patients following cardiac surgery. Twenty-six patients received a loading dose of 100 mg hydrocortisone followed by a continuous infusion of 10 mg/h during postoperative day (POD) 1. Patients received 5 mg/h on POD 2, and dosing was tapered to 20 mg three times a day on POD 3 and 10 mg three times a day on POD 4. Twenty-two comparison patients received standard treatment. Results revealed that the patients who received the hydrocortisone regimen reported significantly fewer PTSD symptoms than comparison patients ($P < 0.05$).

10. Reconsolidation and Tertiary Prevention

An orthodox view of memory formation holds that after the initial labile period has lapsed, and the event has been consolidated into long-term memory, this memory is no longer subject to modification by external agents (although the expression of the memory may be modified, as in extinction). However, the orthodox view has recently been challenged by a series of controversial experiments in animals. In a typical such experiment, an animal is trained in a fear-conditioning task, and the memory is allowed to consolidate. Subsequently, the unreinforced (CS) is presented a single time. Immediately following this, the animal is given a protein synthesis inhibitor. Subsequent testing finds the animal's CR to the CS to be reduced (Nader et al. 2000a). This result has been interpreted as implying that when a previously consolidated memory, or CR, is evoked, i.e., called up from long-term memory storage, it again becomes labile (Nader et al. 2000b). Subsequently, an active process similar to the original consolidation process, requiring new protein synthesis, is required to return this memory to long-term storage. This process is termed "recon-

solidation.”

Whether a protein synthesis inhibitor given following a single CS trial in a previously conditioned animal can really block the reconsolidation of an evoked memory is currently the subject of debate among animal memory researchers. Some studies have failed to find evidence for reconsolidation or its blockade at all (e.g., Cammarota et al. 2004). Even in experiments in which evidence for reconsolidation blockade is found, critics point to the observation that if the animal is followed for a long enough period of time, the CR often reemerges, indicating that the memory has not really been lost (reviewed in McGaugh 2004). This suggests that the protein synthesis inhibitor only temporarily alters the memory's retrievability. However, this latter distinction may be less than critical from the clinical standpoint. It is of sufficient interest that a number of animal experiments have found that a pharmacological intervention during a putative second labile period can reduce the subsequent manifestation of fear CR, at least for some period of time, whether this be due to truly blocked reconsolidation or only to altered retrievability.

From the standpoint of clinical investigation, a practical problem is that the protein synthesis inhibitors used to block reconsolidation in animal experiments are too toxic for human use. Interestingly, however, propranolol may also have the ability to reduce reconsolidation in animals (Debiec and LeDoux 2004; Przybylski et al. 1999), and possibly even humans (Miller et al. 2004).

Several investigators of reconsolidation have not failed to notice its potential therapeutic implications for PTSD (e.g., Debiec and LeDoux 2004; Nader et al. 2000b), although others have discounted any such implication (Izquierdo et al. 2004). Charney (2004) recently hypothesized that, “repeated reactivation and reconsolidation may further strengthen the memory trace and lead to persistence of trauma-related symptoms” (p 204; see *Paradoxical Enhancement/Reconsolidation* loop in Fig. 1). Anything that might give PTSD patients relief from their strong, disturbing traumatic memories, if only temporary, is potentially useful. To investigate this possibility, patients with PTSD who become reexposed to their traumatic CSs, either spontaneously or by therapeutic design, could conceivably be given propranolol, or another suitable agent safe for human use, shortly thereafter, with the goal of blocking the reconsolidation of the evoked traumatic memory and thereby weakening it. Even if the traumatic memory eventually returns, the possibility still exists that it could again be weakened through repeat reconsolidation blockade. Note that this putative intervention would also be mediated by the *Paradoxical Enhancement/Reconsolidation* loop portrayed in Fig. 1. However, in this case, the objective would not merely be to interrupt the positive feedback loop of paradoxical enhancement, but rather to convert it into a negative loop by capitalizing on the theoretical ability of blockade of reconsolidation to weaken an already consolidated memory.

At present, no study of tertiary prevention of PTSD by pharmacological reconsolidation blockade has been reported, although clinical research interest appears to be building. From the paradigmatic standpoint, it could be important to make the traumatic reexposure brief, in contrast to the desirability of making it long in CBT. The reason for this is that if the reexposure were to be long enough to induce ex-

tion, the subsequent pharmacologic intervention could theoretically act to block consolidation of the extinction learning, rather than reconsolidation of the traumatic memory (Eisenberg et al. 2003)

11. Efficacy Versus Effectiveness

This chapter has discussed several promising secondary preventive pharmacological interventions for PTSD, although their efficacy needs to be further tested in larger, randomized controlled trials. It has also touched on possible, novel tertiary preventive intervention, for which no efficacy data yet exist. Even if the efficacy of one or more of these interventions becomes established through further research, this will not equate with effectiveness, which involves the application of the intervention in usual clinical settings. Obstacles to effectiveness include patients' psychological reluctance to accept acute posttraumatic psychiatric interventions, and their proneness to drop out before treatment is completed (Weisaeth 2001). However, this may be less problematic in clinical than in research settings. In research settings involving placebo-controlled trials, the investigator can only offer the patient the possibility of receiving a drug that may be efficacious. Once a drug has proven efficacious in clinical trials, however, the clinician can confidently recommend a drug that is likely to be helpful. Another obstacle is posed by medical contraindications to the administration even of an efficacious drug. Propranolol, for example, may not be able to be safely administered to patients with asthma or heart block, or in situations in which it is clinically necessary to rely upon the sign of tachycardia to disclose the development of a medical complication such as hypovolemia or hypoglycemia. Cortisol may not be able to be given to patients with penetrating wounds that pose a risk of infection. It may turn out, however, that demonstration of the efficacy of one of these, or other, agents would spur the development of new drugs that retain efficacy with fewer contraindications. An example might be a β -blocker with the high capacity to cross the blood-brain barrier of propranolol but lacking in the latter's asthmagenic effect.

12. Conclusion

A recent MEDLINE search on PTSD yielded 8600 articles. Of these, only 5 involved a prospective, controlled, preventive, pharmacotherapeutic clinical trial (Gelpin et al. 1996; Pitman et al. 2002; Schelling et al. 2001; Schelling et al. 2004; Vaiva et al. 2003). Moreover, these studies are properly regarded as preliminary. In a review, Morgan et al. (2003) noted, "To date there is almost no empirical data on effective pharmacological interventions in the immediate aftermath of extreme psychological trauma ... Controlled trials are essential given the limited information in this field" (pp 834, 841).

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