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

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Nobumasa Kato, M.D.
Department of Neuropsychiatry, Faculty of Medicine
The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

Mitsuhiro Kawata, M.D.
Department of Anatomy and Neurobiology
Kyoto Prefectural University of Medicine
Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan

Roger K. Pitman, M.D.
Harvard Medical School
Massachusetts General Hospital
Room 2616, Building 149, 13th Street, Charlestown, MA 02129, U.S.A.

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Preface

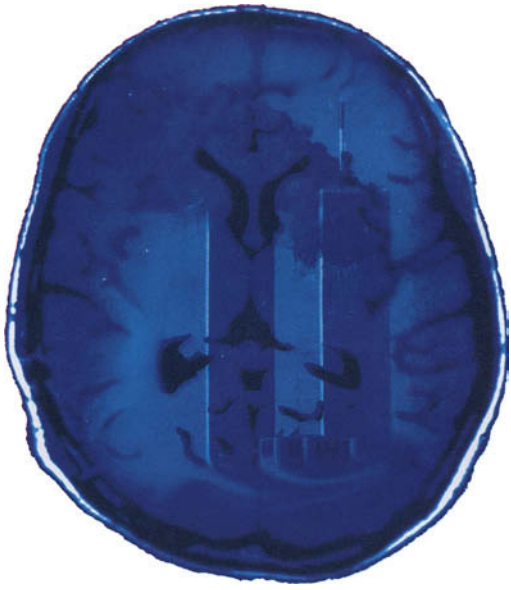
PTSD: A Window into Psychiatric Disorders

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-

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

Contents

Part 2 Clinical Implications for PTSD and Perspectives in Psychiatry

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 maturated 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,

Section of Psychiatry and Behavioral Sciences, Tokyo Medical and Dental University Graduate School, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

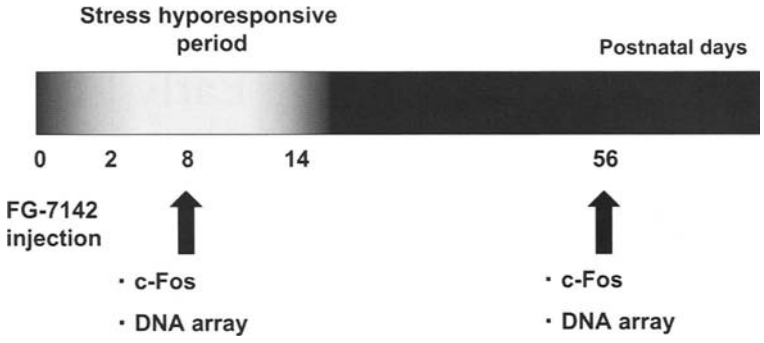


Fig. 1. Schematic representation of the postnatal development of adrenocortical responses to systemic stressors. The stress hyporesponsive period should be noted. The *arrows* indicate the postnatal days for the experiments that examined the regional and developmental differences in the gene expression 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 hyporesponsive 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

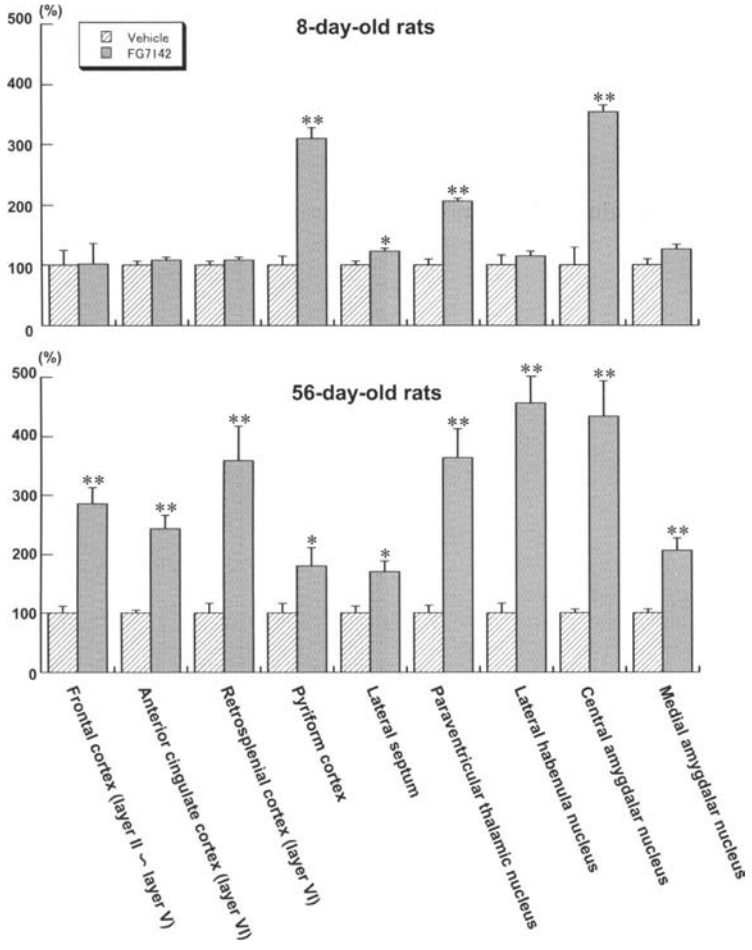


Fig. 2. The density of brain cell nuclei expressing c-Fos-like immunoreactivity in the forebrain of rats at postnatal days 8 and 56 after the systemic administration of FG 7142 (Kurumaji et al. 2003). The rats were treated with FG 7142 (20 mg/kg, i.p.) or vehicle and were perfused 2 h thereafter. The 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 hyporesponsive 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 hyporesponsiveness 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 maturated 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-phospahte 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 hyporesponsive 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 maturate 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.

Department of Medical Pharmacology, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands

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 adrenocorticotropic 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 electrophy siological 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

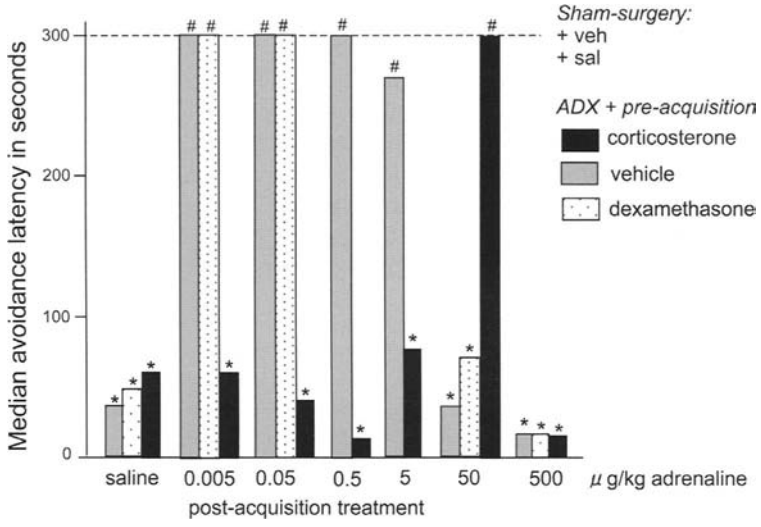

Fig. 1. Retention of step-through inhibitory avoidance response. Corticosterone desensitizes the effect of adrenaline up to ten thousand-fold, dexamethasone is ineffective. Forty-eight hours after adrenalectomy (*ADX*), rats were treated with corticosterone or dexamethasone (300 μg/kg s.c.) 60 min prior 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

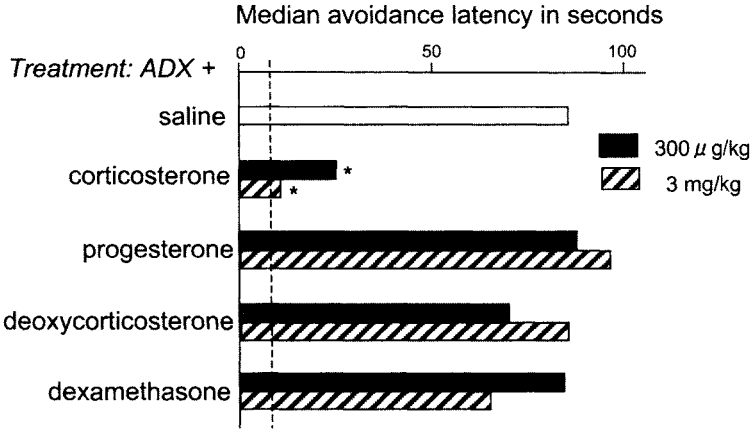

Fig. 2. Corticosterone normalizes extinction behavior. Rats were adrenalectomized and treated with various steroids (300 μg/kg and 3 mg/kg s.c.) 60 min before forced extinction. On the following day, retention of inhibitory avoidance behavior was measured. Forced extinction (exposing the rats for 5 min 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 hRNA 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 clinical 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-

digm 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 (Sutanto 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 allel 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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