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PRENATAL STRESS INDUCES LONG TERM STRESS VULNERABILITY, COMPROMISING STRESS RESPONSE SYSTEMS IN THE BRAIN AND IMPAIRING EXTINCTION OF CONDITIONED FEAR AFTER ADULT STRESS

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Abstract—Stress is a risk factor for the development of affective disorders, including depression, post-traumatic stress disorder, and other anxiety disorders. However, not all individuals who experience either chronic stress or traumatic acute stress develop such disorders. Thus, other factors must confer a vulnerability to stress, and exposure to early-life stress may be one such factor. In this study we examined prenatal stress (PNS) as a potential vulnerability factor that may produce stable changes in central stress response systems and susceptibility to develop fear- and anxiety-like behaviors after adult stress exposure. Pregnant Sprague–Dawley rats were immobilized for 1 h daily during the last week of pregnancy. Controls were unstressed. The male offspring were then studied as adults. As adults, PNS or control rats were first tested for shock-probe defensive burying behavior, then half from each group were exposed to a combined chronic plus acute prolonged stress (CAPS) treatment, consisting of chronic intermittent cold stress (4 °C, 6 h/d, 14 days) followed on day 15 by a single session of sequential acute stressors (social defeat, immobilization, cold swim). After CAPS or control treatment, different groups were tested for open field exploration, social interaction, or cued fear conditioning and extinction. Rats were sacrificed at least 5 days after behavioral testing for measurement of tyrosine hydroxylase (TH) and glucocorticoid receptor (GR) expression in specific brain regions, and plasma adrenocorticotropic hormone (ACTH) and corticosterone. Shock-probe burying, open field exploration and social interaction were unaffected by any treatment. However, PNS elevated basal corticosterone, decreased GR protein levels in hippocampus and prefrontal cortex, and decreased TH mRNA expression in noradrenergic neurons in the dorsal pons. Further, rats exposed to PNS plus CAPS showed attenuated extinction of cue-conditioned fear. These results suggest that PNS induces vulnerability to subsequent adult stress, resulting in an enhanced fear-like behavioral profile, and dysregulation of brain noradrenergic and hypothalamic–pituitary–adrenal axis (HPA) activity.

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Abbreviations: ACTH, adrenocorticotropic hormone; CAPS, chronic plus acute prolonged stress; CORT, corticosterone; GR, glucocorticoid receptor; HPA axis, hypothalamic–pituitary–adrenal axis; HR, high responder rats; ITI, inter-trial interval; LC, locus coeruleus; NE, norepinephrine; PD, postnatal day; PNS, prenatal stress; PTSD, posttraumatic stress disorder; TH, tyrosine hydroxylase; WKY, Wistar-Kyoto rats.

Key words: prenatal stress, traumatic stress, vulnerability, fear extinction, tyrosine hydroxylase, HPA axis.

Affective disorders, such as depression, post-traumatic stress disorder (PTSD) and other anxiety disorders, have long been considered to be stress-related/stress-initiated disorders. Responses to acute stressors are thought to be adaptive in the short term by increasing, for example, access to energy stores, increasing cardiovascular tone, and enhancing behavioral response capabilities. However, when these systems are repeatedly activated, as with chronic stress, dysregulation of a number of hormonal and neurotransmitter systems may occur (McEwen, 2003). Chronic stress is a risk factor, and possibly a causal factor, in the development of depression and anxiety disorders (Kendler et al., 1999; Koenen et al., 2002, 2007; Gilmer et al., 2005; Jordanova et al., 2007). Indeed, a number of physiological and anatomical alterations associated with chronic stress are hallmarks of depression and anxiety disorders (Board et al., 1956; Nemeroff et al., 1984, 1991; Gold et al., 1986; Holsboer et al., 1986; Weisse, 1992; Heuser et al., 1998; Arborelius et al., 1999; Manji et al., 2001; McEwen, 2003). In addition to cumulative or chronic stress, severe acute stress is also associated with mood and anxiety disorders, most prominently with PTSD. (Jordanova et al., 2007; American Psychiatric Association, 2000). However, not all individuals exposed to chronic or acute-traumatic stress in adulthood develop depression or anxiety disorders, suggesting that some other factor or factors, either genetic, epigenetic, or experience-based, contribute to susceptibility to develop affective disorders. Therefore, to fully understand the mechanisms underlying these disorders, it is insufficient to simply examine the response to stressors. Rather, the factors involved in predisposing for failure to recover from the normal response to stress must be identified (Yehuda and LeDoux, 2007).

Early life stress is one potential factor. For example, early life stress is a risk factor for PTSD, specifically, a history of trauma, childhood abuse/neglect, low education and IQ, low socio-economic status, or loss of a parent in childhood (Bremner et al., 1993; Breslau et al., 1999; Widom, 1999; Koenen et al., 2002, 2007). In rodents, prenatal stress (PNS) produces several behavioral and physiological changes that may be indicative of later stress vulnerability (e.g., Weinstock et al., 1992; Valle et al., 1997; Lemaire et al., 2000).

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Two of the most prominent systems involved in stress adaptation, the brain noradrenergic system and the hypothalamic–pituitary–adrenal (HPA) axis, have also been implicated in stress-related pathology. Norepinephrine (NE) is released in response to stress (Morilak et al., 2005; Aston-Jones et al., 1999), chronic stress alters noradrenergic signaling (Buffalari and Grace, 2009; Kitayama et al., 2008; Ma and Morilak, 2005), and noradrenergic dysregulation is reported in numerous affective disorders, including depression and PTSD (Ressler and Nemeroff, 1999; Strawn and Geracioti, 2008). Likewise, the HPA axis is activated in response to acute stress, resulting in release of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) and this response is altered after chronic stress (Dallman, 1993; Ma and Morilak, 2005). Furthermore, HPA axis dysregulation is a consistent component of several affective disorders, including depression, panic disorders, obsessive-compulsive disorder, and PTSD (Nemeroff et al., 1984; Gold et al., 1986; Souetre et al., 1988; Abelson et al., 2007; Kluge et al., 2007; Mason et al., 1986; Pitman and Orr, 1990).

It is being increasingly recognized that changes in executive function and cognitive capability are also prominent features of mood and anxiety disorders (Beck, 1976; Beck et al., 1987; Mathews and MacKintosh, 1998; Coles and Heimberg, 2002). Moreover, in the context of stress, both the brain noradrenergic system and the HPA axis are involved in regulation and dysregulation of cognitive processes such as learning and memory (de Quervain et al., 2009), including specifically conditioned fear and extinction learning (McIntyre et al., 2002; Mueller et al., 2008; Gourley et al., 2009). Impaired cognition, maladaptive fear responses, and impaired extinction of learned fear are primary symptoms of a number of affective disorders, with these fear-related symptoms being most relevant to anxiety disorders such as panic disorder, phobias, obsessive-compulsive disorder, and PTSD (Sutker et al., 1995; Fossati et al., 1999; Koenen et al., 2001; Moritz et al., 2002; Kangaratnam and Asbjørnsen, 2007; Blechert et al., 2007; Wessa and Flor, 2007). Therefore, it is possible that the mechanisms by which vulnerability factors such as prenatal stress may induce long-lasting susceptibility to develop psychopathology upon adult stress exposure could include dysregulation of the HPA axis and/or brain noradrenergic system, resulting specifically in maladaptive responses to fear-provoking stimuli and an impaired ability to extinguish fear responses in non-stressful conditions.

Thus, the purpose of the present study was to examine neurobiological correlates of adult stress vulnerability induced by PNS exposure. We measured the effects of PNS followed by a combined chronic plus acute prolonged stress (CAPS) treatment as adults, on tyrosine hydroxylase (TH) expression in the locus coeruleus (LC) and adrenal medulla, HPA status, and glucocorticoid receptor (GR) protein levels in the prefrontal cortex (PFC) and hippocampus. In the same rats, we also tested the vulnerability of PNS-exposed adult rats to develop fear or anxiety-like behaviors following exposure to CAPS treatment, on measures of acute stress reactivity, social interaction, fear conditioning, and extinction. We hypothesized that PNS exposure would produce stable, long-term changes in central and peripheral stress response systems, and a vulnerability to subsequent adult stress exposure such that the behavioral impact of adult stress would be greater. Portions of this work have been presented in abstract form (Green et al., 2010).

EXPERIMENTAL PROCEDURES

Animals

Timed-pregnant (6 days pregnant upon arrival) female Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) were singly housed throughout pregnancy. On postnatal day (PD) 5, litters were culled to eight pups each, maximizing the number of males retained (typically, three to six per litter), and weaned on PD 21. Upon weaning, male pups were pair-housed with a littermate until PD 41–45, depending on the experiment, at which time they were singly housed prior to starting the adult stress or unstressed control treatments. The rats were housed in Plexiglas cages ($25\times45\times15$ cm^3) on a 12/12 h light-dark cycle (lights on at 7:00 h) with food and water available *ad libitum*. In total, 141 adult male offspring (from 63 litters—33 stressed and 30 unstressed) were used in these experiments. In addition, for the social defeat procedure, six adult male Long–Evans rats (Harlan), weighing at least 400 g, were used as defeaters. They were housed, together with an ovariectomized female, in large resident cages ($80\times55\times40$ cm^3) in a separate room on the same 12/12 h light cycle. All experiments were conducted during the light phase. All procedures were conducted according to NIH guidelines for the care and use of laboratory animals and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio. All efforts were made to minimize animal pain, suffering or discomfort, and to minimize the number of rats used.

Prenatal stress treatment

After 1 week in the housing facility, half of the pregnant females were immobilized daily for 1 h, from day 14 of pregnancy until birth (8–9 days). Immobilization involved taping the rat's torso and limbs gently but snugly in a prone position on a flat platform, allowing no movement. Unstressed control pregnant females were left undisturbed during this same time period.

Shock-probe defensive burying test

At PD 41–43, a subset of the offspring ($n=60$) from both groups were tested in the shock probe defensive burying test. This was to evaluate potential differences in active and passive behavioral stress-reactivity as a consequence of the prenatal stress treatment prior to any exposure to adult stress. The rats were placed into a modified cage containing 5 cm of bedding, with a shock probe protruding 6 cm into one end of the cage. The probe was set to deliver 2 mA of current when the probe was touched. After the rat made contact with the probe and received a shock, the current was shut off and the 15 min test began. Behavior was recorded using a CCD camera mounted above the cage and stored to video files for offline scoring and analysis. The dependent measures analyzed were the amount of time spent immobile and the amount of time spent engaged in actively burying the probe. After the shock-probe defensive burying test, these animals were individually housed. Likewise, rats not tested in the shock-probe defensive burying test were also individually housed at this same time point.