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HOUSING ENVIRONMENT MODULATES PHYSIOLOGICAL AND BEHAVIORAL RESPONSES TO ANXIOGENIC STIMULI IN TRAIT ANXIETY MALE RATS

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Abstract—Environmental enrichment can modulate mild and chronic stress, responses to anxiogenic stimuli as well as drug vulnerability in a number of animal models. The current study was designed to examine the impact of postnatal environmental enrichment on selectively bred 4th generation high- (HAn) and low-anxiety (LAn) male rats. After weaning, animals were placed in isolated (IE), social (SE) and enriched environments (EE) (e.g., toys, wheels, ropes, changed weekly). We measured anxiety-like behavior (ALB) on the elevated plus maze (EPM; trial 1 at postnatal day (PND) 46, trial 2 at PND 63), amphetamine (AMPH) (0.5 mg/kg, IP)-induced locomotor behavior, basal and post anxiogenic stimuli changes in (1) plasma corticosterone, (2) blood pressure and (3) core body temperature. Initially, animals showed consistent trait differences on EPM with HAn showing more ALB but after 40 days in select housing, HAn rats reared in an EE showed less ALB and diminished AMPH-induced activity compared to HAn animals housed in IE and SE. In the physiological tests, animals housed in EE showed elevated adrenocortical responses to forced novel object exposure but decreased body temperature and blood pressure changes after an air puff stressor. All animals reared in EE and SE had elevated brain-derived neurotrophic factor (BDNF)-positive cells in the central amygdala (CeA), CA1 and CA2 hippocampal regions and the caudate putamen, but these differences were most pronounced in HAn rats for CeA, CA1 and CA2. Overall, these findings suggest that environmental enrichment offers benefits for trait anxiety rats including a reduction in behavioral and physiological responses to anxiogenic stimuli and AMPH sensitivity, and these responses correlate with changes in BDNF expression in the central amygdala, hippocampus and the caudate putamen.

Key words: environmental enrichment, elevated plus maze, heart rate, body temperature, BDNF, central amygdala.

INTRODUCTION

Anxiety disorders are prevalent and have been studied extensively in clinical settings (Young et al., 2001; Mojtabai et al., 2002; Ressler and Mayberg, 2007). Many people suffering from anxiety often also present with substance use (Merikangas et al., 1998) with an estimated 17.71% of people meeting criteria for both a 12-month substance use disorder and anxiety disorder (Grant et al., 2004). An often-used method for studying anxiety in an animal model is the exploitation of selective breeding to produce animals that display a specific anxiety profile. Inbred lines of high anxiety-like behavior (HAB) rats show similar profiles to anxious clinical populations, with increased adrenocortical response (Landgraf et al., 1999) and greater activation in brain areas implicated in anxiety (Salomé et al., 2004; Hasler et al., 2007). Individuals abusing psychostimulant drugs such as cocaine also show compromised adrenocortical responses, indicating that dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis implicated in anxiety disorders, may contribute to aspects of addiction as well (Contoreggi et al., 2003). Rats phenotyped as high responders (e.g., rats that self-administer amphetamine (AMPH) and sucrose more readily) (Piazza et al., 1989) show a more robust corticosterone (CORT) response to novelty exposure (Cain et al., 2005). Further, in Lewis and Fischer 344 inbred rat strains that show varying vulnerabilities to psychostimulant drugs of abuse, baseline CORT levels in a novel environment are positively correlated with AMPH locomotor activity (LMA) (Miserendino et al., 2003).

While adrenocortical responses are an index of HPA axis activity (for review, see Lovallo, 2006), with persistent stress elevating plasma CORT levels (Brennan et al., 2000), physiological changes such as

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Abbreviations: ACTH, adrenocorticotropic hormone; ALB, anxiety-like behavior; AMPH, amphetamine; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CeA, central amygdala; CORT, corticosterone; CPu, caudate putamen; DAP, diastolic arterial pressure; EE, enriched environment; EPM, elevated plus maze; HAn, high anxiety; HPA, hypothalamic–pituitary–adrenal; IE, isolated environments; LAn, low anxiety; LMA, locomotor activity; MAP, mean arterial pressure; OA, open arm; PND, postnatal day; SAP, systolic arterial pressure; SE, social environment; SEM, standard error of the mean.

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body temperature and heart rate have also been shown to fluctuate in rodents following mild environmental stressors such as odors, air puffs and strobe lights (Harkin et al., 2002). Psychological stressors can also interact with these measures, with early work by Long and colleagues (1990) showing that ''cage-switch stress'' (moving a rat to an empty cage previously occupied by another rat) increased body temperature on average by 1.21 °C. More recent work has also shown increases in body temperature (0.93 °C) and heart rate in rats following a brief air puff (Harkin et al., 2002), and stress-induced hyperthermia (Olivier et al., 2003). Still other mild stressors such as novel odors or strobe lights can elevate body temperature, basal heart rate, and increase LMA in rodents (Harkin et al., 2002). Differences in physiological measures have been noted in animals exhibiting anxiety-like behavior in social defeat paradigms (Bhatnagar et al., 2006) and in rats exposed to acute and repeated social stress paradigms, with tachycardia responses showing adaptation to repeated stress (Chen and Herbert, 1995). Inhibition of cellular activity with gamma aminobutyric acid (GABA) in the dorsomedial hypothalamus attenuates the adrenocorticotropic hormone (ACTH), tachycardia and blood pressure spikes while GABA stimulation in the paraventricular nucleus only affected ACTH levels following the air stress (Stotz-Potter et al., 1996).

The enriched environment (EE), social housing cages equipped with toys that provide sensorimotor stimulation, has been implicated in restoring stress-induced learning deficits and depressive-like behavior (Cui et al., 2006). Enrichment can also modify the brain and adrenocortical response to stress (Diamond, 2001) and sensitivity to psychostimulant drugs of abuse (Bardo et al., 2001) in a number of animal models. Further, higher basal levels of ACTH have been found in males reared in isolated environments (IEs) versus those reared in group housing. In addition, following stress exposure, those in isolation also exhibited an increase in CORT and ACTH compared to their group-housed counterparts (Weiss et al., 2004). There are also a few studies examining the impact of EE on the physiological responses to stress that show EE can reverse elevated heart rate, systolic blood pressure and hyperthermia (Lawson et al., 2000; Sharp et al., 2005).

Neurotrophin expression, particularly brain-derived neurotrophic factor (BDNF) expression, has been implicated in the benefits associated with environmental enrichment (Ickes et al., 2000; Rossi et al., 2006). Furthermore, BDNF expression is thought to influence synaptic modifications that may underlie the neuroplasticity necessary for stress resilience since BDNF knock-out mice are stressed (for review, see Chourbaji et al., 2008), and BDNF heterozygous mice only show a *partial* recovery in exploratory behavior and dendritic spine proliferation from EEs compared to their wild-type counterparts (Zhu et al., 2009).

The current study was designed to determine how EE might influence basal and mild stress-induced physiological responses (e.g., CORT, heart rate, blood pressure and core body temperature) in selective outbred animals phenotyped as high (HAn) and low anxiety (LAn) (i.e., unrelated mating pairs). In addition, we set out to assess any changes in anxiety response on the elevated plus maze (EPM) and AMPH-induced locomotion in HAn/LAn animals following EE, social environment (SE) and IE. Finally, we measured brain-derived neurotrophin factor (BDNF) protein levels in the hippocampus, central amygdala (CeA) and caudate putamen (CPu) to assess any changes in levels depending on trait anxiety or postnatal housing experience.

EXPERIMENTAL PROCEDURES

Experimental subjects

Sixty male Long Evans rats were used in this study. All animals were acquired from the fourth generation of HAn or LAn unrelated same-phenotype pairings. These trait anxiety lines were bred at the University of Massachusetts Boston taking care to not cross sibling pairs. HAn or LAn status was determined using percent open-arm (OA) time and percent OA entries in the EPM with LAn animals showing less anxiety-like behavior (upper quartile) in the apparatus than their HAn counterparts (lower quartile). Animals were maintained in a temperature and humidity controlled environment on a 12-h light–dark cycle (lights on at 800 h); food and water were available *ad libitum* except during testing procedures. All protocols received approval from the University of Massachusetts IACUC and closely followed the applicable portions of the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23; Revised 1996).

Postnatal housing environments and timing of testing

On average postnatal day (PND) 23, animals were weaned and not more than two male littermates were used to establish the trait and postnatal housing environment groups. A total of 30 LAn males were placed in either an isolated (IE, $n = 10$), social (SE, $n = 10$), or enriched environment (EE, $n = 10$). The same groups were established using 30 HAn males ($n = 10$ per group). The IE consisted of one rat housed in a standard Plexiglas cage ($17 \times 24 \times 20$ cm) with contact bedding. The SE was constructed of a large Plexiglas rectangular environment ($24 \times 40 \times 81$ cm) with normal contact bedding housing ten same-phenotyped animals. The EE consisted of a large wire metal cage with a smooth metal floor ($94 \times 94 \times 51$) equipped with contact bedding. Various objects consisting of plastic and wood toys (e.g. Lego blocks, buckets, rattles, wheels, hides) as well as objects to promote movement such as ropes, hanging ladders and hanging chains were included and rearranged and/or changed weekly with new objects being introduced or others removed. Animals remained in IE, SE and EE for a minimum of 40 days and then began a schedule of behavioral and physiological tests with allowance for rest periods between tests (Fig. 1).