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SHORT-TERM AND LONG-TERM EFFECTS OF REPEATED SOCIAL DEFEAT DURING ADOLESCENCE OR ADULTHOOD IN FEMALE RATS

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Abstract—Accumulating evidence suggests that adolescence represents a sensitive period during which social stressors influence adult behavior and stress reactivity. However, relatively little is known about the impact of social stress in adolescence on behaviors or stress reactivity in females. In this study, we exposed adolescent or adult female rats to the repeated social stress of defeat for seven consecutive days. Repeated defeat resulted in distinctly different behavioral repertoires during defeat in adolescent compared to adult female rats. Adolescent females exhibited more play and avoidant behaviors and adult females exhibited more active and aggressive behaviors toward the resident female. Examination of the short-term effects of social defeat using the Porsolt forced swim test (FST) indicated that adolescents, regardless of their exposure to social defeat, showed increased time immobile and decreased time swimming compared to adults. Adolescent rats exposed to defeat also exhibited increased climbing compared to their age-matched naïve counterparts. These effects dissipated with age. Interestingly, no effects of defeat were observed in adult females, however, when these females were re-assessed in the FST 30 days after the end of defeat, we observed increased swimming at the expense of climbing. Using exposure to a novel restraint to assess stress reactivity, we found that stress during adolescence and adulthood led to lower basal adrenocorticotropic hormone concentrations and that both stressed and control adolescent groups exhibited a delay in recovery in adulthood compared to stressed and control adult groups. Fos protein analysis further suggested that cortical/thalamic structures serve as potential substrates that mediate these long-term impacts of stress during adolescence. Thus, repeated social stress during adolescence produces different patterns of effects as compared with repeated social stress during adulthood.

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Abbreviations: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; EDTA, ethylenediaminetetraacetic acid; FST, forced swim test; HPA, hypothalamic–pituitary–adrenal; PFC, prefrontal cortex; PVN, paraventricular nucleus of the hypothalamus; PVT, paraventricular nucleus of the thalamus; TBS, tris-buffered salaine.

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Key words: social stress, resident intruder, adolescent, Porsolt forced swim test, restraint.

INTRODUCTION

Exposure to stressful life events is a risk factor for the onset of a variety of disorders during adulthood (McEwen, 2003; Wood et al., 2010). Adolescence, which is a time of rapid cerebral development and organization most notably in the hippocampus, prefrontal cortex, and amygdala (Spear, 2000; Romeo et al., 2006), may be a particularly vulnerable time for the effects of stress. Repeated social stress has been shown to impact the brain norepinephrine system that regulates defensive strategies (Bingham et al., 2011), increasing adolescents' susceptibility to anxiety and depression (Garber, 2006; Romeo et al., 2006; Weintraub et al., 2010). Passive coping in response to stress has been associated with greater activation of the hypothalamic–pituitary–adrenal (HPA) stress axis (Walker et al., 2009), which may increase the risk of depression, whereas proactive coping in response to stress has been associated with higher sympathetic activity and resiliency (Billings and Moos, 1984). Differential effects of stress in adolescent as compared with adult animals suggest a window of potential plasticity in the HPA stress response and imply that the impact of stress may not be the same at all ages of development (McCormick and Mathews, 2010; Romeo, 2010; Foilb et al., 2011; Jankord et al., 2011). Similarly, prior stress history may increase or decrease the magnitude of physiological stress responses (Grissom et al., 2008), and different experimental stressors may result in different behavioral outcomes as well as different HPA and neural reactivity profiles (Grissom et al., 2007).

Studies in animals have also suggested that stress during adolescence has a particularly strong effect in female rats, with adult females isolated in adolescence exhibiting increased stress reactivity (Weintraub et al., 2010). Adult female rats exposed to social stress in adolescence exhibited increased startle reflex suggesting an anxiety-like phenotype (Bourke and Neigh, 2012) whereas adult female rats subjected to chronic mixed-modality stress did not exhibit an affected startle response compared to age-matched controls (Bourke

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and Neigh, 2011). Social isolation stress has also been shown to produce an active coping behavioral phenotype in adult female rats but not in adult male rats assessed in the forced swim test (FST) (Hong et al., 2011). Evidence from studies using physical restraint stress have shown a pattern of delayed recovery for female relative to male rats (Romeo, 2010) and have suggested that females are more vulnerable than males to the physiological effects of chronic restraint (Campbell et al., 2003). However, little is known about the impact of repeated social defeat during adolescence, whether it produces different outcomes in the short- and long-term and how its effects are different than social defeat experienced in adulthood.

The resident-intruder model of defeat (Miczek, 1979) has been shown to be a useful model for studying the impact of repeated social stress in rats. In this model a rat (intruder) is placed in the home cage of a larger, aggressive rat (resident) and subjected to repeated threatening encounters. Adult male rats exposed to this type of social defeat stress have been shown to exhibit decreased motivation (Becker et al., 2008) and to experience altered HPA function (Bhatnagar and Vining, 2003), which may promote depressive-like behaviors (Miczek et al., 2004). Although many modifications to the resident-intruder paradigm have been implemented, repeated resident-intruder stress during adolescence seems to increase proactive responses in the FST for early adolescent male rats (Bingham et al., 2011), whereas it tends to induce a shift from active to passive coping behavior in adult female rats (Bourke and Neigh, 2011). It is not known whether social defeat stress alone can impact the development of affect-related behaviors differently in the immediate term (during adolescence) compared to the long-term (adulthood) in female rats. In this study, we examined the impact of chronic social defeat stress on behavior during defeat in adolescent or adult females, and assessed the short and long-term effects on behavioral coping, HPA reactivity, and neural activation in these animals.

EXPERIMENTAL PROCEDURES

Animals and housing

The experimental animals consisted of female Sprague–Dawley rats ($n = 49$) that were purchased from Charles River Laboratories (Wilmington, MA, USA) at PND 29-31 (early adolescence) or at PND 69–71 (adulthood). Animals were randomly assigned to stress (experimental) and naïve (control) groups and run in four separate cohorts (final n's of Adolescent Stress = 15; Adolescent Naïve = 7; Adult Stress = 19; Adult Naïve = 8). The specific group sizes for each measure are provided in the figure legends and can vary from measure to measure particularly in the neuroendocrine/neural measures due to lack of sample or elimination of outliers (samples more than two standard deviations from the mean). All animals were pair-housed within same-age, same-treatment sub-groups in standard 26 cm × 46 cm clear polypropylene cages with *ad libitum* access to food and water. Animals were allowed to acclimate to a 12-h light/dark cycle (lights on at 06:00 AM), temperature-controlled standard colony room for approximately 1 week prior to study initiation. A group of 10 Sprague–Dawley lactating adult rats were housed separately for use as resident animals in the resident-intruder test to induce the stress of defeat. These females were housed in cages that were 48 cm × 35 cm × 20 cm (width × depth × height) in size. All procedures were conducted between 9:00 and 11:00 AM. The experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Children's Hospital of Philadelphia Research Institute's Animal Care and Use Committee.

Resident-intruder social stress paradigm

The resident-intruder social stress paradigm (Miczek, 1979) was used with the modification that lactating female rats were used as resident animals because lactating females are likely to defeat other female rats (Flannelly and Flannelly, 1987). On each of seven consecutive days, adolescent or adult experimental animals (intruders) were subjected to 30-min episodes of social defeat stress. Intruders were individually placed in the home-cage of a novel lactating female (resident) whose pups had been removed immediately prior. Following the intruder's placement in the resident's cage, resident and intruder were allowed to interact until one of two possible criteria was met: (a) the intruder exhibited a submissive defeat posture (>2 s frozen in a supine position), or (b) 15 min. elapsed (as in our previous study with adult male rats (Wood et al., 2010)). Upon reaching one of these criteria, the animals were separated by a wire barrier, allowing only auditory, olfactory and visual contact for the remainder of the 30-min test period. Intruders were then returned to their home-cages and lactating mothers (residents) were reunited with their pups. The social defeat occurred at the same time each day with the intruder being randomly placed into the cage of a different lactating female each day. Naïve animals remained in their home-cages in the colony room for the duration of the 30-min test.

All 30-min social defeat episodes were videotaped and later scored by two trained investigators who were blind to the experimental conditions. Videotapes were evaluated to record the timing of each attack on the intruder, the number of attacks received, and latency to assume a submissive posture or defeat (if applicable). In addition, to document the development of submission in intruder animals, frequencies of each of the following six behaviors were tallied: *'boxing'* (intruder assumed posture on hindlegs while in physical contact and attentive to the resident); *'upright posture'* (intruder assumed posture on hindlegs while attentive toward the resident but with no physical contact); *'under the cover'* (intruder was underneath the resident); *'grooming by resident'* (intruder was aggressively groomed by the resident), *'freezing/crouching posture'* (intruder assumed a completely still and crouching posture in response to resident's presence); *'self-grooming'* (intruder groomed itself during the interaction time).

Short-term and long-term behavioral effects of repeated social stress

To assess the immediate and long-term behavioral responses to repeated social defeat stress, all experimental animals were individually subjected to the Porsolt FST on two occasions. Based on the work of Lucki (1997), the two-day FST involved a 15-min forced swim (on day 1), followed 24 h later by a 5-min FST (on day 2). The 5-min swim test was videotaped from directly above the clear glass cylinder [46 cm in height × 20 cm in diameter], filled to 35 cm with water at a temperature of 25 °C (± 1 °C). The first Porsolt FST occurred on the 2 days following the seventh social defeat episode (day 8–9), after which rats were removed from the water, towel-dried, placed back into their home-cages and left undisturbed for 30 days. The cylinder was cleaned and the water was changed before testing of the next subject. To assess the long-term behavioral effects of repeated social defeat, experimental animals were