Hormones and Behavior 93 (2017) 31–38

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

An acute social defeat stressor in early puberty increases susceptibility to social defeat in adulthood

Anna M. Rosenhauer *, Katharine E. McCann, Alisa Norvelle, Kim L. Huhman

Neuroscience Institute, Georgia State University, 161 Jesse Hill Jr. Drive, Atlanta, GA 30303, USA

ARTICLE INFO

Article history:
Received 11 January 2017
Revised 21 March 2017
Accepted 4 April 2017
Available online xxxx

Keywords:
Development
Sex differences
Aggression
Conditioned defeat

ABSTRACT

Syrian hamsters readily display territorial aggression. If they lose even a single agonistic encounter, however, hamsters show striking reductions in aggressive behavior and increases in submissive behavior, a distinct behavioral change that we have previously termed conditioned defeat. This acute social defeat stressor is primarily psychological and is effective in both males and females. Therefore, we maintain that this procedure presents an ideal model for studying behavioral and physiological responses to social stress. Here, we demonstrate that social avoidance following social defeat is a particularly useful dependent measure because of its sensitivity and stability between sexes and across the estrous cycle. In addition, we demonstrate that peripubertal hamsters exposed to a single, 15 min social defeat exhibit significantly more social avoidance 24 h later when compared with no-defeat controls. Later, defeated and non-defeated hamsters display similar agonistic behavior in adulthood indicating that the peripubertal defeat does not alter adult territorial aggression. After experiencing an additional social defeat in adulthood, however, the hamsters that experienced the pubertal defeat respond to the adult defeat with increased social avoidance when compared with hamsters that were defeated only in adulthood and with no-defeat controls. These data are the first to show that a single social defeat in puberty increases susceptibility to later social defeat in both males and females.

1. Introduction

Exposure to social stress has been implicated in the etiology of a variety of neuropsychiatric disorders including mood and anxiety disorders and posttraumatic stress disorder (PTSD) (Dobry et al., 2013; Kessler et al., 1997; Saveanu and Nemeroff, 2012; van Winkel et al., 2013; Wood and Bhatnagar, 2015). Social stress is the most common stressor that humans encounter (Björkqvist, 2001; Heim and Nemeroff, 2001; Kessler, 2003; Vasconcelos et al., 2015), and humans perceive social stressors to be more intense than non-social stressors (Björkqvist, 2001). Our lab has developed a model of social defeat using Syrian hamsters. Both male and female Syrian hamsters are generally aggressive and will readily defend their territory against intruders, even in the laboratory (Albers et al., 2002; Gattermann et al., 2001). Hamsters rapidly and reliably establish stable dominant-subordinate relationships during a brief social interaction, and the agonistic behaviors that are produced to establish these hierarchical relationships are a potent stressor to the loser in the encounter (Huhman et al., 2003, 1992, 1991, 1990). The ritualized nature of hamster agonistic behavior offers the distinct advantage in that brief encounters rarely result in any physical injury or trauma. Furthermore, even a single social defeat in this species has prominent and long-lasting behavioral effects including a striking elimination of aggressive behavior accompanied by a concomitant increase in submissive behavior and social avoidance, a response that we have termed conditioned defeat (Huhman, 2006; McCann et al., 2014; McCann and Huhman, 2012; Potegal et al., 1993).

In addition to the ease and rapidity with which conditioned defeat can be elicited in hamsters, another distinct advantage of using a hamster social defeat protocol is that the behavioral manipulations and testing can be done similarly in both males and females (Huhman et al., 2003; Solomon et al., 2007a). This is particularly important because stress-induced disorders, and mental illness in general, often present with sexually dimorphic symptoms and prevalence (Boyd et al., 2015; Maeng and Milad, 2015). Previous work from our lab has demonstrated that there is a sex difference in the behavioral response to social defeat when hamsters are subsequently tested with a non-aggressive intruder (NAI), with defeated females displaying considerably less submission toward the NAI (Huhman et al., 2003). We have also demonstrated that female responses to defeat vary depending on gonadal hormone status (Faruzzi et al., 2005; Solomon et al., 2007b). More recently, rodent social defeat models have focused on social avoidance, operationally defined as the time spent in the opposite side of the cage from a confined conspecific, as a measure of stress-induced social anxiety

* Corresponding author.
E-mail addresses: arosenhauer1@student.gsu.edu (A.M. Rosenhauer), kmccann3@gsu.edu (K.E. McCann), anorvelle@gsu.edu (A. Norvelle), khuhman@gsu.edu (K.L. Huhman).

http://dx.doi.org/10.1016/j.yhbeh.2017.04.002
0018-506X/

(Berton et al., 2006; Haller and Bakos, 2002; McCann and Huhman, 2012). This dependent measure is easy to quantify and reduces the variability in behavior that a freely moving conspecific (e.g., NAI) can introduce to an agonistic encounter. Thus, the purpose of Experiment 1 was to compare social avoidance in male and female hamsters following an acute social defeat to determine if the previously reported sex difference in agonistic behavior that has been observed in response to an NAI is also evident when the opponent is confined to a small box within the testing arena. In Experiment 2, we tested the hypothesis that social avoidance of a caged opponent in females varies over phases of the estrous cycle.

All of our previous social defeat work has been done in adults. It is thought, however, that stressful experiences may be particularly impactful during earlier periods of development. A growing body of evidence indicates that stress encountered during early development has long-lasting neurobiological effects (for review see, Boersma et al., 2014; Heim et al., 2010; Nemeroff, 2016) and is linked to increased likelihood of maladaptive responses to stress in adulthood (Maccari et al., 2014; Patchev et al., 2014). Puberty has also been increasingly recognized as an additional sensitive period during which exposure to stress may be especially impactful (Andersen and Teicher, 2008; Brown and Spencer, 2013; Holder and Blaustein, 2014; Romeo, 2010). In hamsters, the effects of chronic social subjugation during the pubertal period have been examined in males and females (Bastida et al., 2009; Delville et al., 1998; Taravosh-Lahn and Delville, 2004; Wommack et al., 2004; Wommack and Delville, 2007). In contrast to the conditioned defeat response observed in adult hamsters, chronic social subjugation throughout puberty in males hastens the development of adult patterns of aggression and enhances aggressiveness in adulthood. It is not known, however, whether exposure of male or female hamsters to an acute social defeat during puberty has any short- or long-term effects. Thus, in Experiment 3 we examined first whether an acute social defeat in peripubertal males and females increases social avoidance behavior as it does in adults, and, next whether this early experience of defeat alters either adult territorial aggression or behavioral sensitivity to a later social defeat.

2. Methods

2.1. Animals

For Experiments 1 and 2, male and female Syrian hamsters (*Mesocricetus auratus*) approximately 12 weeks of age were obtained from Charles River Laboratories (Wilmington, MA). These animals were singly housed and weighed 120–140 g at the beginning of the experiments. Animals in Experiment 3 were bred in-house from stock obtained from Charles River. Litters were left undisturbed with their dam and littermates until weaning at post-natal (PN) day 25 when juveniles were singly housed. Animals for all experiments were housed in polycarbonate cages (23 × 43 × 20 cm) with wire lids, corncob bedding, and cotton nesting material in a temperature-controlled colony room on a 14:10 light/dark cycle. Food and water were available ad libitum throughout all studies. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Resident aggressors (RAs) used for social defeat and social avoidance testing were larger, adult hamsters that were singly housed for at least one month and that reliably responded with aggression toward same-sex intruders placed in their home cages. Male RAs were used to defeat all male subjects. Female RAs that were used to defeat female subjects were ovariectomized to prevent variation in RA aggression across the estrous cycle. In Experiment 3, nonaggressive intruders (NAIs) used as stimulus animals for adult agonistic behavior testing were smaller, adult hamsters that were group housed. Again, animals were paired with a same-sex opponent. NAIs of either sex do not generally display any aggressive behavior upon being placed into another hamster's home cage. Experimenters monitored the estrous cycle of female NAIs, and no NAIs were used for testing on the day of Estrus.

2.2. Behavior

2.2.1. Groups

Timelines for each experiment are shown in Fig. 1. Phase of the estrous cycle was determined via vaginal swabs for 8 consecutive days to ensure stable estrous cycles in all adult females. Males were also handled each day during estrous monitoring. In Experiment 1, animals (males, $n = 16$; females, $n = 19$) were divided by sex, weight-matched, and then randomly assigned to a defeat condition (defeat or no-defeat). Thus, the four groups formed a 2 (defeat) × 2 (sex) design. In Experiment 2, animals (males, $n = 16$; females, $n = 63$) were assigned to groups representing each day of the estrous cycle: Diestrus Day 1 (D1), Diestrus Day 2 (D2), Proestrus (P), and Estrus (E), weight-matched, and then randomly assigned to a defeat or no-defeat condition. Weight-matched males were tested each day that females were tested for comparison and to control for possible random effects that might occur on different testing days. In Experiment 3, animals (males, $n = 42$; females, $n = 39$) were divided by sex and litter and then assigned to one of three, weight-matched groups (puberty and adult defeat, adult defeat only, and no-defeat control) at weaning. Note that to ensure the reproducibility and reliability of these results, this experiment was completed twice with the additional home-cage/no-defeat control group included in the second iteration, as reflected in group *n*'s above (individual group *n*'s are shown in figures). Estrous cycles of adult females were monitored via vaginal swabs for 8 days before aggression testing occurred on approximately PN64 (±3 days to account for cycle because testing occurred on D2). Again, the same number of males was tested each day along with the females.

2.2.2. Social defeat training

All behavioral manipulations took place within the first 2 h of the dark phase of the light:dark cycle to minimize potential circadian variation in the data. Animals were transported to the behavior suite and were given at least 30 min to habituate to the environment. Defeat training occurred during a 15 min exposure to the home cage of a same-sex RA, as described previously (Huhman et al., 2003). The holding box used for social avoidance testing, described below, was placed in the cage during training for habituation to the apparatus, and a clear cage top was placed on top of cages during training and testing to prevent escape. RAs attacked intruders, including those in early puberty, within approximately 30 s of the subject's placement in the aggressor's cage. In Experiment 1, females were defeated on D2 and were tested on P based on our previous finding that this timing results in the most pronounced conditioned defeat in females (Solomon et al., 2007b). In Experiment 2, we also observed, however, that no-defeat controls tested on P showed increased social avoidance and that avoidance behavior in females most closely resembled that produced by males when females were tested on D2 (see Results); therefore, all females were defeated on D1 and tested on D2 in Experiment 3. All defeat training and subsequent testing was done under dim red illumination and was recorded with a CCD camera for later behavioral scoring as described below. No-defeat controls were also transported to the behavior suite and were exposed to an empty cage with bedding from a same-sex RA's cage for 15 min. Home cage controls in Experiment 3 remained undisturbed in the colony room during pubertal manipulations and served as no-defeat controls, described above, in adulthood.

2.2.3. Social avoidance testing

In all experiments, social avoidance testing (duration 5 min) took place 24 h after defeat training, as described previously (McCann and Huhman, 2012). Briefly, defeated and non-defeated hamsters were returned to the behavior suite as on the previous day and were placed