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Corticosterone may interact with peripubertal development to shape adult resistance to social defeat

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ABSTRACT

Studies of social stress in adult mice have revealed two distinct defeat-responsive behavioral phenotypes; "susceptible" and "resistant," characterized by social avoidance and social interaction, respectively. Typically, these phenotypes are observed at least 1 day after the last defeat in adults, but may extend up to 30 days later. The current study examined the impact of *peripubertal* social defeat on immediate (1 day) and adult (30 day) social stress phenotypes and neuroendocrine function in male C57BL/6 mice. Initially, peripubertal (P32) mice were resistant to social defeat. When the same mice were tested for social interaction again as adults (P62), two phenotypes emerged; a group of mice were characterized as susceptible evidenced by significantly lower social interaction, whereas the remaining mice exhibited normal social interaction, characteristic of resistance. A repeated analysis of corticosterone revealed that the adult (P62) resistant mice had elevated corticosterone following the social interaction test as juveniles. This was when all mice, regardless of adult phenotype, displayed equivalent levels of social interaction. Peripubertal corticosterone was positively correlated with adult social interaction levels in defeated mice, suggesting early life stress responsiveness impacts adult social behavior. In addition, adult corticotropin-releasing factor (CRF) mRNA in the paraventricular nucleus of the hypothalamus (PVN) was elevated in all defeated mice, but there were no differences in CRF mRNA expression between the phenotypes. Thus, there is a delayed appearance of social stress-responsive phenotypes suggesting that early life stress exposure, combined with the resultant physiological responses, may interact with pubertal development to influence adult social behavior.

1. Introduction

Adolescence is a critical developmental period during which social stress can have lasting emotional and behavioral consequences (Buwalda et al., 2011; Paus et al., 2008; Spear, 2000). Several animal studies demonstrate a significant impact of early life stress exposure on neuroendocrine function and behavioral outcomes. For example, a single exposure to elevated platform stress as a juvenile combined with adult swim stress increases anxiety-like behavior as measured by open-field and acoustic startle (Avital and Richter-Levin, 2005). Thus, juvenile stress exacerbates the consequences of adult stress. Similarly, a 3 day stress exposure in adolescent rats (PND 34, 45 or 55) reduces locomotion and increases acoustic startle when animals are tested as adults (Cymerblit-Sabba et al., 2015). Other studies show that juvenile and adult neuroendocrine responses to stress are different. Juvenile exposure to several acute stress procedures increases adrenocorticotropic hormone (ACTH) and corticosterone secretion that persists for twice as long compared with adult rats (Goldman et al., 1973; Romeo et al., 2006b; Romeo et al., 2006c; Romeo et al., 2004; Vázquez and Akil, 1993). Exposure to chronic restraint stress, however, produces an elevated corticosterone response in juveniles and a faster return to baseline compared to adults (Romeo et al., 2006b). The latter effect is due to increased activation of corticotropin-releasing factor (CRF) neurons in the paraventricular nucleus (PVN) of the hypothalamus in juveniles compared with adults, and suggests pubertal maturation reorganizes neuroendocrine stress responses (Romeo et al., 2006b).

Although these studies have uncovered important differences in physiological and behavioral responses to non-social stress between juveniles and adults, they provide little information on specific effects of juvenile *social* stress on these outcomes. Studies in Syrian hamsters show that males exposed to repeated social stress as juveniles display an accelerated transition of agonistic behavior from play fighting to adult aggression (Wommack and Delville, 2003; Wommack et al., 2003). This effect is due, in part, to elevated secretion of glucocorticoids (Wommack and Delville, 2007; Wommack et al., 2005), suggesting that early hypothalamic-pituitary-adrenal (HPA) axis function can shape adult behavior. It is not completely clear if a more social species, such as mice, show similar alterations in aggression in response to juvenile or pubertal social stress. The limited studies available find that defeated juvenile or pubertal mice display reduced social interaction, increased

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anxiety-like behavior, and spatial learning deficits as adults (Jacobson-Pick et al., 2011; Novick et al., 2013; Vidal et al., 2007).

Most social stress studies in mice focus on adults and have revealed two distinct defeat-responsive behavioral phenotypes; "susceptible" and "resistant" (aka unsusceptible or resilient) characterized by social avoidance and social interaction, respectively. These phenotypes are observed at least 1 day after the last defeat session, but may extend up to 30 days later (Dulka et al., 2015; Gilman et al., 2015; Krishnan et al., 2007; Meduri et al., 2013). In addition, although a large literature has identified several neurobiological mechanisms underlying susceptibility and resistance to social defeat in adult animals (Berton et al., 2006; Cao et al., 2010; Gilman et al., 2015; Jasnow et al., 2004a; Jasnow et al., 1999; Jasnow et al., 2004b; Jasnow and Huhman, 2001; Jasnow et al., 2005; Krishnan et al., 2007; Romeo et al., 2007; Vialou et al., 2010) few studies examine the ontogeny of these behavioral phenotypes by documenting the longitudinal effects of juvenile social stress.

In the current set of experiments we examine the immediate and long term effects of peripubertal social stress in mice and focus on identifying predictive markers and mechanisms underlying the divergent phenotypic behavioral responses to peripubertal social defeat that are present in adult mice. Specifically, we exposed peripubertal male mice to mild repeated social defeat followed by social interaction tests 1 day and 30 days later. We measured serum corticosterone and testosterone as well as brain CRF mRNA levels to identify behavioral, physiological, and molecular indicators associated with adult social defeat-responsive behavioral phenotypes.

2. Methods

2.1. Animals

All mice were male C57BL/6 mice bred in our animal facility and weaned 21 days after birth (P21). Experimental C57BL/6 mice were group housed (2–5 per cage) with male littermates until the first day of defeats, after which they were singly housed for the duration of the experiment (32 days). Animals were left undisturbed throughout the duration of behavioral testing except for routine animal care. Male CD — 1 mice used as aggressors were individually housed and prescreened for aggression. Group housed, age-matched male CD — 1 mice were used as social targets during social interaction. All mice were housed on a 12:12 light:dark cycle with lights on at 7 a.m. and allowed access to food and water *ad libitum*. Experiments and procedures were approved by Kent State University Institute of Animal Care and Use Committee (IACUC) and conducted in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals, 8th Ed.

2.2. Experiment 1: longitudinal behavioral responses to peripubertal social defeat

Thirty-three C57BL/6 mice were used in Experiment 1. Experimental mice were housed and weaned as described above and were exposed to 2 days of social defeat or control procedures and tested for social avoidance behavior 1 day (P32) and again 30 days (P62) after the last day of defeat.

2.2.1. Social defeat procedure

P28–31 male C57BL/6 was randomly assigned to treatment groups (control or defeat). Social defeat involved placing the experimental mouse into the home cage of an aggressive, territorial CD — 1 mouse for 5 min or 3 attacks, whichever came first (modified to "3 attacks" from Meduri et al., 2013). The number of attacks was recorded to ensure that each experimental mouse was attacked equally. Additionally, we have previously shown that animal-to-animal variability in attacks does not influence the development of susceptible and resistant phenotypes (Meduri et al., 2013). Following exposure to attacks, mice were separated across a Plexiglas divider for the remainder of an hour for sensory exposure to the CD — 1 mouse and their cage (Fig. 1B). After the 55-minute separation, mice were transferred into the cage of a different CD — 1 mice and the defeat was repeated until each mouse encountered 4 different aggressors per day for 2 consecutive days. Control mice were placed across a Plexiglas divider for an entire hour with no physical contact. Social defeat occurred between 11 a.m. and 5 p.m.

2.2.2. Social interaction procedure

Experimental mice were tested in social interaction approximately 24 h after the last social defeat procedure and within 4 h of the onset of the dark cycle (P30–33) (referred to as P32). These same experimental mice were tested again 30 days after the initial interaction test (P60–63) (referred to as P62; Fig. 1A). During each social interaction test, animals were placed in an open field arena (46 cm × 46 cm × 39 cm; Coulbourn Instruments) where social behavior was measured under dim red light. A Galaxy cup with metal bars (Spectrum, Streetsboro, OH; Moy et al., 2004; Nadler et al., 2004) was placed upside-down against one of the four corners. The metal bars are spaced to allow sensory (olfactory, visual and auditory), but not physical, contact. During the first trial, experimental mice were placed into the center of the arena and allowed to explore for 300 s. During the second trial, social

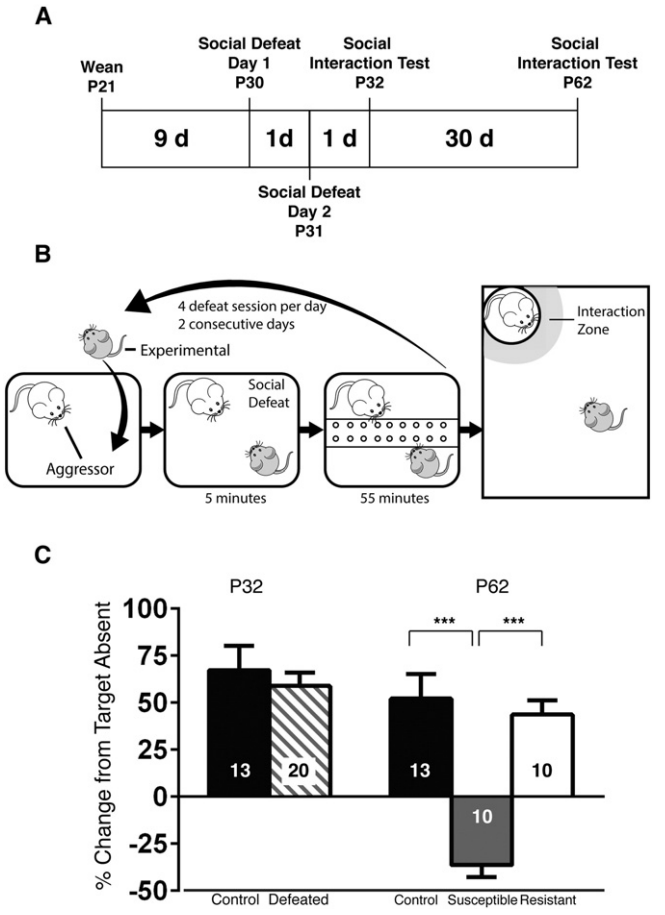

Fig. 1. Peripubertal social defeat results in a delayed emergence of susceptible and resistant phenotypes. (A) Timeline for peripubertal social defeat (P30) and social interaction (P32), as well as adult social interaction tests (P62). (B) Schematic representation of the social defeat procedure and social interaction test. (C) At P32, peripubertal defeated mice interact with a target mouse at similar levels compared with controls ($p > 0.05$). The same mice tested again as adults at P62 display two behavioral phenotypes. A subset of mice exhibit a susceptible phenotype characterized by significantly less interaction compared to controls ($p < 0.001$) whereas the remainder exhibit a resistant phenotype characterized by social interaction similar to non-defeated controls. Control and resistant mice interact at a similar level ($p > 0.05$). Data are expressed as mean ± SEM. $^{***}p < 0.001$.