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Deficits in male sexual behavior in adulthood after social instability stress in adolescence in rats

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ABSTRACT

There is increasing evidence that exposure to stressors in adolescence has long-lasting effects on emotional and cognitive behavior, but little is known as to whether reproductive functions are affected. We investigated appetitive and consummatory aspects of sexual behavior in male rats that were exposed to chronic social instability stress (SS, n = 24) for 16 days in mid-adolescence compared to control rats (CTL, n = 24). Over five sexual behavior test sessions with a receptive female, SS rats made fewer ejaculations ($p = 0.02$) and had longer latencies to ejaculation ($p = 0.03$). When only data from rats that ejaculated in the fifth session were analyzed, SS rats (n = 18) had reduced copulatory efficiency (more mounts and intromissions before ejaculation) compared to CTL rats (n = 19) ($p = 0.004$), and CTL rats were twice as likely as SS rats to make more than one ejaculation in the fifth session ($p = 0.05$). Further, more CTL (14/24) than SS (5/25) rats ejaculated in four or more sessions ($p = 0.05$). SS rats had lower plasma testosterone concentrations than CTL rats ($p = 0.05$), but did not differ in androgen receptor, estrogen receptor alpha, or Fos immunoreactive cell counts in the medial preoptic area. The groups did not differ in a partner preference test administered between the fourth and fifth sexual behavior session. The results suggest that developmental history contributes to individual differences in reproductive behavior, and that stress exposures in adolescence may be a factor in sexual sluggishness.

Introduction

Changes in the reactivity of stress systems, notably the hypothalamic–pituitary–adrenal (HPA) axis, during times of biological transition are thought to confer vulnerability to the organism (Dorn and Chrousos, 1997). Nevertheless, less is known about the stress-related plasticity of the adolescent period than is known for other stages of ontogeny. Although the onset and offset of adolescence is gradual, there are qualitative differences in behavior between adolescence and both earlier and later stages of life that highlight the significant reorganization of brain function that occurs during that period of development (reviewed in Doremus-Fitzwater et al., 2010; McCormick and Mathews, 2010). The adolescent brain may be particularly vulnerable to the effects of stressors for many reasons. First, because of a heightened rate of development in adolescence compared to adulthood, the brain may be more malleable during that time (reviewed in Brenhouse and Andersen, 2011; Crews et al., 2007). Second, the HPA axis functions differently in adolescence, with a more prolonged release of glucocorticoids in response to a stressor in adolescent rats compared to adult rats (reviewed in Romeo, 2010); glucocorticoids affect brain development and plasticity, and underlie several "programming" effects of environmental experiences in early life (Harris and Seckl, 2011; Seckl, 2008). Third, stressors affect gonadal function, which also plays a role in adolescent brain development. For example, there are organizational effects of gonadal hormones in adolescence that serve to shape male social behavior and associated neural circuitry (e.g., Hebbard et al., 2003; Schulz et al., 2004). Thus, the effects of stressors on brain development may be mediated in part by effects on the gonadal system. Most of the research investigating the consequences of stress exposures in adolescence, however, has focused on endpoints such as cognitive and emotional function (reviewed in McCormick and Green, in press; McCormick et al., 2010), with few studies investigating reproductive function.

In the few available studies, there is mixed evidence regarding the effects of stressors in adolescence on male reproductive function. Although some chronic stressors administered in periadolescence decreased circulating testosterone concentrations (Llorente et al., 2011; Retana-Marquez et al., 2003), repeated immobilization stress in adolescence increased testosterone concentrations (Almeida et al.,

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2000a, 2000b). Chronic isolation housing in adolescence typically impaired sexual behavior (e.g., Bulygina et al., 2005; Ward and Reed, 1985), although one study found that isolation housing beginning in early adolescence improved sexual performance of male rats compared to pair-housed rats (Swanson and van de Poll, 1983). Detrimental effects of such lengthy social deprivation are not limited to adolescence, however; chronic isolation housing beginning in adulthood also impaired sexual performance of male rats (Brotto et al., 1998). Subjugation stress in adolescence in hamsters decreased the latency to mount receptive females (Ferris et al., 2005), but had little effect on sexual behavior in rats (Frahm et al., 2011). Rats that underwent 6 h of daily immobilization stress for 15 days at postnatal day 40 had longer latencies to mount, but an increased frequency of thrusting when tested soon after the last stress exposure (which the authors characterized as better sexual performance); neither the stressed nor control rats ejaculated during the session (Almeida et al., 2000a). Furthermore, most experiments included only one sexual behavior test session, and thus whether group differences would increase or decrease with more experience with receptive females is unknown.

Here, we investigated whether social instability stress experienced in mid-adolescence in males would impair sexual behavior when tested six weeks after the last stress exposure in adulthood. The social instability stress (SS) procedure (daily 1 h isolation followed by change of cage partner) was administered in mid-adolescence from postnatal days 30 to 45, and thus spans pre- and post-pubertal periods as defined by balanopreputial separation (which occurs at about 40–42 days of age), although plasma concentrations of testosterone are still significantly lower at postnatal day 45 than in adulthood (>postnatal day 60) (reviewed in McCormick and Mathews, 2010). Sexual performance was measured in five sessions with a receptive female to investigate whether differences between adolescent SS and control rats increased or decreased with experience. Between the fourth and fifth session, a partner preference test was administered to assess sexual motivation (e.g., Harding and Velotta, 2011; Kelliher and Baum, 2001; Vagell and McGinnis, 1997). We previously found that as adults, male SS rats do not differ from control male rats in social approach (time spent near novel male confined behind wire mesh), but they spent less time engaged in social interactions when given access to a novel male than did control rats (Green et al., in press). Thus, we hypothesized that SS in adolescence may affect sexual performance rather than sexual motivation. Because adolescent stressors have been found to decrease testosterone concentrations, and male sexual behavior involves testosterone's actions at androgen receptors (AR) and, through its aromatization to estradiol, at estrogen receptors (ER) (reviewed in Baum, 2003), we measured testosterone concentrations before the first and after the last sexual behavior test session.

We also investigated after the last test session whether SS and control rats differed in Fos expression in the medial preoptic area (mPOA), a critical neural region for male sexual performance (reviewed in Sakamoto, 2012). Although several neural regions show increased expression of Fos after male sexual behavior, Fos expression in the mPOA is specific to performance aspects (mounts and intromission) rather than motivational aspects such as anogenital investigation, with expression of Fos proportional to the amount of sexual activity (reviewed in Coolen, 2005). In parallel sections, we investigated whether SS and control rats differed in the number of cells expressing AR and ERα in the mPOA; because AR gene expression in the MPOA increase during adolescence (Walker et al., 2009), effects of adolescent stressors on sexual behavior may involve effects on receptor expression.

Methods

Animals

Male Long Evans rats (n = 48) arrived at 22 days of age and female rats (n = 32) arrived later at 55 days of age from Charles Rivers Laboratories (St. Constant, Quebec). All rats were housed in same condition pairs and placed on a 12 h light–dark cycle (lights on at 08:00 h). Food and water was made available ad libitum. All experimental procedures complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985) and the Canadian Council on Animal Care guidelines and received approval from the Brock University Animal Care and Use Committee.

Social stress procedure

Rats were given 7 days to acclimate to the colony, and males were randomly assigned to either the social stress (SS, n = 24) or the no stress control (CTL, n = 24) conditions. Every day from 30 to 45 days of age, SS rats were isolated in a room outside the colony, each in a small ventilated container (12 cm in diameter) for 1 h. After isolation, rats were returned to the colony and paired with a new cage partner from the SS condition such that they were never housed with the same partner twice. The stress procedure was performed during the lights on phase and at various times throughout the day to minimize predictability and habituation. CTL rats were not disturbed except for feeding and cage maintenance. This stress procedure is known to result in an elevated exposure to glucocorticoids compared to that in control rats and in rats that are exposed to daily isolation only in the absence of the social instability (absence of change in cage partners) (McCormick et al., 2007). Further, although this stress procedure produced immediate and delayed (evident several weeks after the stress exposure) effects on cognitive performance (McCormick et al., 2012; Morrissey et al., 2011), the same stress exposure administered to adult males produced neither immediate nor delayed effects compared to control males (Morrissey et al., 2011); thus, this stress procedure allows investigation of stress-related plasticity that may be unique to the adolescent period (see also a review of the model in McCormick, 2010).

Ovariectomy and hormone regimen of females

Female rats were ovariectomized at about 65 days of age approximately two weeks before sexual behavior test sessions. Females were first anesthetized with a ketamine (40–50 mg/kg) and xylazine (6–8 mg/kg) cocktail administered s.c. Their sides were shaved and disinfected with Betadine and 75% ethanol. Ovaries were accessed through bilateral dorsolateral incisions and were removed after ligation of the fallopian tubes. Incisions were sutured, and recovery was monitored.

Any given female was used for sexual behavior testing only once within a 4 day period. To induce sexual receptivity, females were injected with 10 μg estradiol benzoate (Sigma) suspended in 100 μl of sesame oil 48 h before, and 0.5 mg of progesterone (Sigma) suspended in 200 μl of sesame oil 3.5 h before participating in two 45 min test sessions.

Sexual behavior testing

Before testing began, males were dummy-coded so that behavior would be scored blind to experimental group. Test sessions were conducted in a separate room from the colony under red light and were video-recorded. Sessions were conducted between 1 and 4 h after lights off (12/12 light cycle). To allow testing to be restricted to these hours, the experiment was run with two cohorts of rats containing equal numbers of CTL and SS rats, and each cohort was divided into subgroups of 8 (4 CTL and 4 SS), with only one subgroup tested on any given day. Thus, on a given test day there were 8 test sessions involving pairings of 8 males with 4 females. Two rats (pairs from the same cage) were tested at the same time in separate