Hormones and Behavior xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Hormones and Behavior

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

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

Cheryl M. McCormick [a,b,*], Matthew R. Green [a], Nicole M. Cameron [c], Feather Nixon [a], Marisa J. Levy [c], Rachel A. Clark [c]

[a] Department of Psychology, 500 Glenridge Ave., St. Catharines, Ontario, Canada L2S 3A1
[b] Centre for Neuroscience Brock University, 500 Glenridge Ave., St. Catharines, Ontario, Canada L2S 3A1
[c] Department of Psychology, Center for Developmental and Behavioral Neuroscience, Binghamton University, Binghamton, NY 13902, USA

ARTICLE INFO

Article history:
Received 6 September 2012
Revised 4 November 2012
Accepted 9 November 2012
Available online xxxx

Keywords:
Stress
Adolescence
Reproductive behavior
Testosterone
Partner preference test
Copulatory behavior
Sexual motivation

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.,

* Corresponding author at: Department of Psychology and Centre for Neuroscience, Brock University, 500 Glenridge Ave., St. Catharines, Ontario, Canada L2S 3A1. Fax: +1 905 688 6922.
E-mail address: cmccormick@brocku.ca (C.M. McCormick).

0018-506X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yhbeh.2012.11.009

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

Please cite this article as: McCormick, C.M., et al., Deficits in male sexual behavior in adulthood after social instability stress in adolescence in rats, Horm. Behav. (2012), http://dx.doi.org/10.1016/j.yhbeh.2012.11.009

chambers to prevent any effect of sequential removal of a cage partner on behavior during the test sessions. Males were habituated to one of two test chambers for 10 min, twice, 1 and 2 days before testing commenced (Table 1 for a timeline of experimental procedures). Males were paired with a different female in each of the five sessions. After the first sexual behavior test session, males were housed individually to prevent aggressive interactions.

The number and timing of mounts without intromissions (mounts), intromissions, and ejaculations were scored. Once these data were collected, subsequent analyses were performed to determine the latencies to display a mount, intromission, and ejaculation. When a rat did not engage in a behavior, a maximum score of 2700 s was given for the latency; this latency corresponds to the total length of the session. The average number of mounts and intromissions required to reach an ejaculation and the refractory period (recovery phase after an ejaculation) were also calculated.

To obtain a score that reflects the sexual behavior of each rat in each session, rats were assigned a score of 0 if they did not mount, intromit, or ejaculate, 1 if they only mounted, 2 if they mounted and intromitted, 3 if they ejaculated, 4 if they ejaculated twice, and 5 if they ejaculated three times in a session.

Receptive vs. non-receptive female partner preference test

The preference test was used to assess sexual motivation (e.g., Harding and Velotta, 2011; Kelliher and Baum, 2001; Vagell and McGinnis, 1997). The apparatus consisted of an open compartment made of white melamine (60 cm × 30 cm × 60 cm) with two small boxes (30 cm × 30 cm × 30) at each end. Wire mesh separated the small boxes from the larger compartment. A hormone-primed, receptive female was placed in one small box, and a non-receptive female was in the other small box. A male rat was then placed in the center of the compartment for a 15 min session. Videotracking software was used to measure the percentage of time in the zone (one third of compartment) near the receptive female and the zone (one third of compartment) near the non-receptive female. The partner preference test was conducted during the dark phase of the light cycle under red light. To minimize any effect of heightened anxiety in SS rats (Green et al., in press; McCormick et al., 2008) on performance, and to allow motivation to be assessed after some sexual experience and yet allow brains to be collected for Fos-immunoreactive cell counts in response to sexual behavior after the fifth sexual behavior test session, the test was conducted between the fourth and fifth sessions.

Table 1
Experimental timeline.

Age (days)	Procedure
22	Housed in pairs in colony.
30 to 45	SS: daily 1 h isolation and re-housed with new cage partner. CTL: undisturbed except for cage maintenance.
46 to 84–86	SS: housed with original partner; undisturbed except for cage maintenance. CTL: undisturbed except for cage maintenance.
83	Blood sample collected.
84–85, 85–86, or 86–87	Two days of habituation (10 min) to the test chamber.
86, 87, or 88	First sex behavior test session of 45 min. Singly-housed after test session for remainder of the experiment.
89, 90, or 91	Second sex behavior test session of 45 min.
92, 93, or 94	Third sex behavior test session of 45 min.
95, 96, or 97	Fourth sex behavior test session of 45 min.
98, 99, or 100	Receptive vs. non-receptive partner preference test.
101, 102, or 103	Fifth sex behavior test session of 45 min. Anesthetized and perfused 1 h after the session for immunohistochemistry. A blood sample was obtained at the onset of perfusion.

Testosterone

A blood sample (approximately 200 μl) was obtained from a tail nick from the rats at 83 days of age, before sexual behavior testing began. A second sample was obtained at the time of perfusion after the last test session. Blood samples were collected in ice chilled tubes containing EDTA and were centrifuged at 1730 ×*g* and 4 °C for 10 min. Plasma was collected and stored at −20 °C until testosterone was measured using enzyme-linked immunosorbent assay kits (Neogen, Lansing MI) and a Biotek Synergy plate reader. The cross-reactivity of the antibody is 100% with testosterone and dihydrotestosterone, and less than 1% with other steroids. Testosterone was extracted from the 50 μl samples using ethyl ether and the samples were reconstituted in 100 μl of the buffer provided in the kit, then further diluted (20 μl in 980 μl buffer). Duplicate aliquots (50 μl) of the final dilution were used for the assay. Intra-assay variance was 6.3% and inter-assay variance was 13.8%. Assay sensitivity is 0.006 ng/ml.

Immunohistochemistry

Two hours after the beginning of the last sex behavior test session (45 min in test session and 75 min in home cage), rats were deeply anesthetized with Euthanyl® and perfused transcardially with 0.9% saline and 4% paraformaldehyde in a 0.1 M phosphate buffer (pH 7.4). Brains were removed from the skulls and kept in 30% sucrose and paraformaldehyde solution until equilibrated. Coronal sections (40 μm) were taken throughout the mPOA using a cryostat (ThermoShandon) and were kept in cryoprotectant at −20 °C until the time of the assay. Immunohistochemistry was performed on three parallel sets of four sections (sections within a set were separated by 240 μm), one set for the detection of Fos, one for the detection of AR, and one for the detection of ERa.

Sections were generously washed in 0.1 M phosphate buffered saline (PBS; pH 7.4), then washed in PBS-X (PBS with 0.3% Triton X-100). Sections were incubated at room temperature in a 0.3% hydrogen peroxide solution for 30 min and were washed in PBS-X, then blocked in 10% goat serum (Sigma) solution for 1 h. Sections were incubated at room temperature overnight in primary antibody (either 1:2000 c-Fos rabbit polyclonal IgG, sc-52; AR rabbit polyclonal IgG, AR sc-816; or ERα rabbit polyclonal IgG, sc-542; all from Santa Cruz Biotechnology). The next day, sections were washed in PBS-X, then incubated for 2 h at room temperature in secondary antibody (biotinylated goat anti-rabbit IgG, Vector Laboratories; 1:500). After another series of washes in PBS-X, sections were incubated in avidin:biotinylated enzyme complex (Vector Laboratories) for 1.5 h at room temperature. Sections were again washed in PBS-X, and then incubated at room temperature for 5 min in diaminobenzidine and nickel staining solution (see DAB SK-4100 instructions; Vector Laboratories). After a final series of washes in PBS-X, sections were mounted on Superfrost plus slides (Fischerbrand) and coverslipped the next day.

Immunostained sections were viewed using a using Nikon digital camera attached to an Eclipse brightfield microscope and Nikon ACT-1 software. Pictures of the mPOA were taken between bregma −0.12 mm and −0.48 mm (according to Paxinos and Watson, 2005). Immunoreactive (ir) cells were counted at 400× magnification in a 250 μm^2 area in each hemisphere. No ir cells were detected in control sections that were not treated with primary antibody. The mean number of ir cells per hemisphere per rat per primary antibody type was used for analysis.

Statistical analyses

Statistical analyses were performed using SPSS version 19 software and consisted of independent group t-tests, and mixed-factor

Please cite this article as: McCormick, C.M., et al., Deficits in male sexual behavior in adulthood after social instability stress in adolescence in rats, Horm. Behav. (2012), http://dx.doi.org/10.1016/j.yhbeh.2012.11.009

(Group × Session, for sexual behavior test measures or Group × Side for partner preference test measures) analysis of variance (ANOVA), Chi squares, and Pearson correlations. An alpha level of $p<0.05$, two-tailed, was used to determine statistical significance.

Results

In brief, rats of both groups improved sexual performance across sessions. There was evidence of deficits in sexual performance in SS rats compared to CTL rats; the main differences between SS and CTL rats were that SS rats had longer latencies to ejaculation, made fewer ejaculations, had lower sex scores, and lower testosterone concentrations than did CTL rats.

Sexual performance measures in each session

Fig. 1 shows the measures obtained for mounts, intromissions, and ejaculation in each session for both groups.

Mounts

The number of mounts increased across sessions ($F_{4,184}=7.92$, $p<0.0001$, partial $\eta^2=0.15$). The groups did not differ in number of mounts ($F_{1,46}=2.62$, $p=0.11$, partial $\eta^2=0.05$), and there was no interaction of Group and Session ($F_{4,184}=1.54$, $p=0.19$, partial $\eta^2=0.03$). The effect of Group in latency to mount was not significant ($F_{4,184}=2.24$, $p=0.14$, partial $\eta^2=0.05$), and there was no effect of Session ($F_{4,184}=1.39$, $p=0.24$, partial $\eta^2=0.03$) or interaction of Group and Session ($F_{4,184}=1.33$, $p=0.26$, partial $\eta^2=0.03$).

Intromissions

The number of intromissions increased across sessions ($F_{4,184}=8.70$, $p<0.0001$, partial $\eta^2=0.16$). The effect of Group was not significant ($F_{1,46}=3.50$, $p=0.07$, partial $\eta^2=0.07$), and there was no interaction of Group and Session ($F_{4,184}=1.02$, $p=0.40$, partial $\eta^2=0.02$). The latencies to intromission of CTL rats and SS rats were not significantly different ($F_{1,46}=3.26$, $p=0.08$, partial $\eta^2=0.07$). Latencies decreased across sessions ($F_{4,184}=6.26$, $p<0.001$, partial $\eta^2=0.12$), and there was no interaction of Group and Session ($F_{4,184}=1.63$, $p=0.17$, partial $\eta^2=0.03$).

Ejaculations

The number of ejaculations in a session increased across days ($F_{4,184}=10.26$, $p<0.0001$, partial $\eta^2=0.18$). CTL rats made more ejaculations than SS rats ($F_{1,46}=5.77$, $p=0.02$, partial $\eta^2=0.11$),

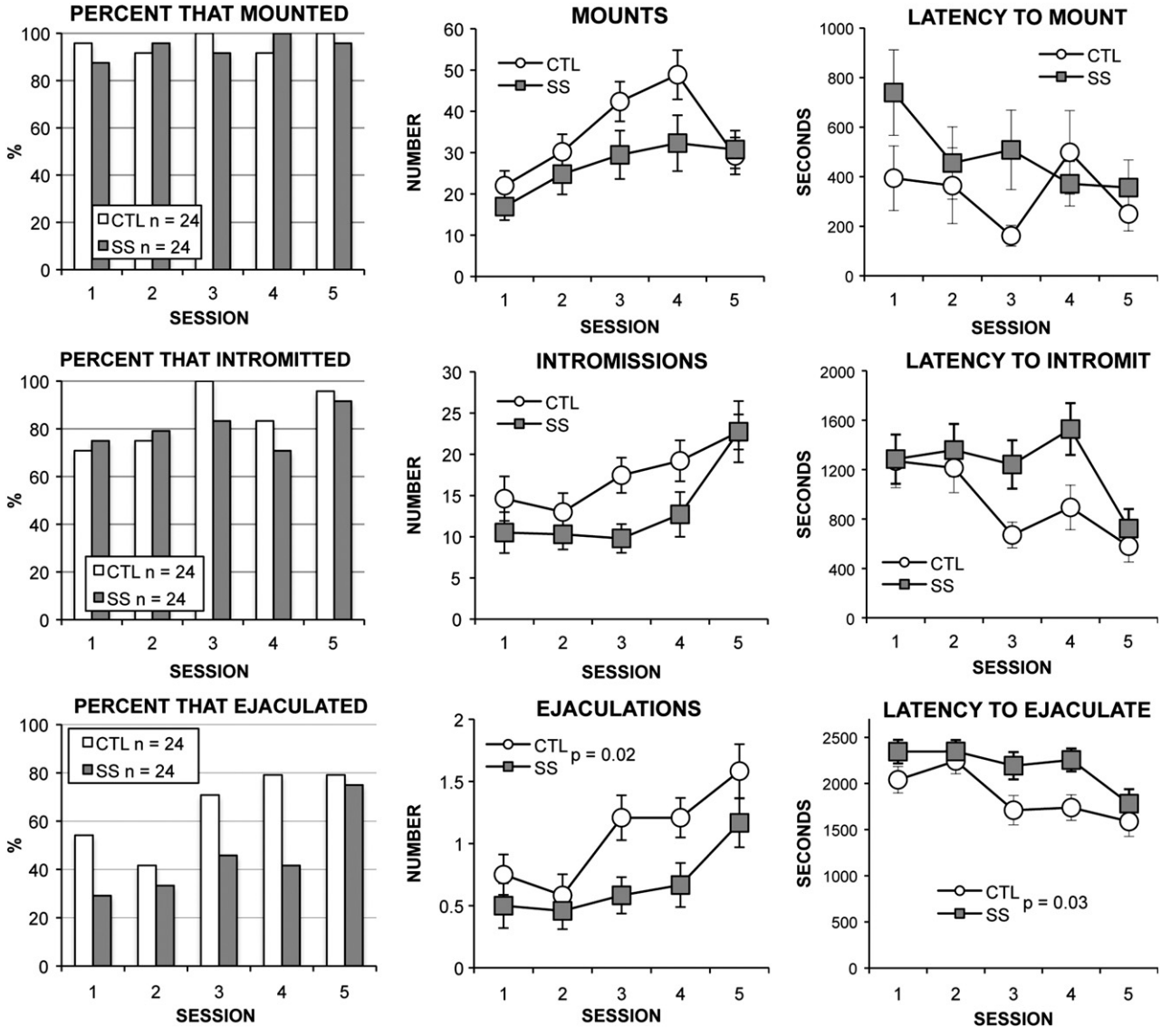

Fig. 1. Percentage of SS and CTL rats that mounted, intromitted, and ejaculated as well as the mean (±S.E.M.) number of, and latency to perform, mounts, intromissions, and ejaculations in each session.

Please cite this article as: McCormick, C.M., et al., Deficits in male sexual behavior in adulthood after social instability stress in adolescence in rats, Horm. Behav. (2012), http://dx.doi.org/10.1016/j.yhbeh.2012.11.009

and there was no interaction of Group and Session ($F_{4,184}=.98$, $p=0.42$, partial $\eta^2=0.02$). The mean number of ejaculations made in the five sessions by CTL rats was 5.33 (±0.60) and by SS rats was 3.34 (±0.55). The latency to ejaculation was shorter for CTL rats than for SS rats ($F_{1,46}=5.31$, $p=0.03$, partial $\eta^2=0.10$), latencies decreased across sessions ($F_{4,184}=8.59$, $p\leq0.001$, partial $\eta^2=0.15$), and there was no interaction of Group and Session ($F_{4,184}=1.23$, $p=0.30$, partial $\eta^2=0.02$).

Every rat except for three SS rats completed at least one ejaculatory sequence in the five sessions. The number of sessions in which rats made an ejaculation was higher for CTL rats than for SS rats ($t_{46}=2.08$, $p=0.04$, Cohen's $d=0.61$). More CTL (14/24) than SS (5/24) rats ejaculated in 4 of the five sessions ($\chi^2=4.15$, $p=0.05$) (Fig. 2).

Copulatory efficiency in the fifth session

Of those that ejaculated in the last session, the mean ejaculations made by CTL rats (2±0.17; n=19) did not differ from SS rats (1.56±0.18, n=18) ($t_{35}=1.77$, $p=0.09$, Cohen's $d=0.59$) (Fig. 3). The number of mounts and intromissions to ejaculation were higher in SS rats than in CTL rats ($t_{35}=3.09$, $p=0.004$, Cohen's $d=1.04$) (Fig. 3). For those rats for which a refractory period could be calculated (i.e., rats that mounted female after an ejaculation: n=17 CTL rats; n=12 SS rats) the length of the refractory period did not differ for SS and CTL rats (Fig. 3). 58% (14/24) of CTL rats and 29% (7/24) of SS rats made more than one ejaculation in the fifth session ($\chi^2=4.15$, $p=0.05$).

Sex scores

There was an effect of Session for sex scores ($F_{4,184}=9.42$, $p<0.001$, partial $\eta^2=0.17$), with higher sex scores obtained in later sessions (Fig. 4). CTL rats had higher sex scores than SS rats ($F_{1,46}=4.29$, $p=0.04$, partial $\eta^2=0.09$), and there was no interaction of Group and Session ($F_{4,184}=1.09$, $p=0.36$, partial $\eta^2=0.02$).

Female partner preference test

A Group×Side ANOVA on percentage of time spent near the receptive female compared to the non-receptive female found no significant preference for the side near the receptive female ($F_{1,46}=3.20$, $p=0.08$, partial $\eta^2=0.07$), no Group differences ($F_{1,46}=0.14$, $p=0.85$, partial $\eta^2=0.007$), nor an interaction of Group and Side ($F_{1,46}=1.00$, $p=0.32$, partial $\eta^2=0.02$) (Fig. 5).

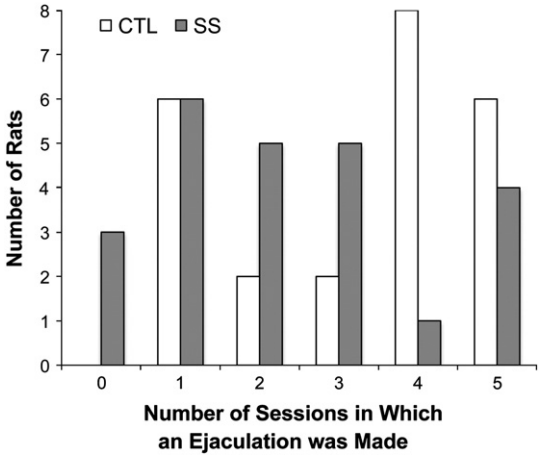

Fig. 2. Frequency distribution showing the number of sessions in which an ejaculation occurred for both SS and CTL rats.

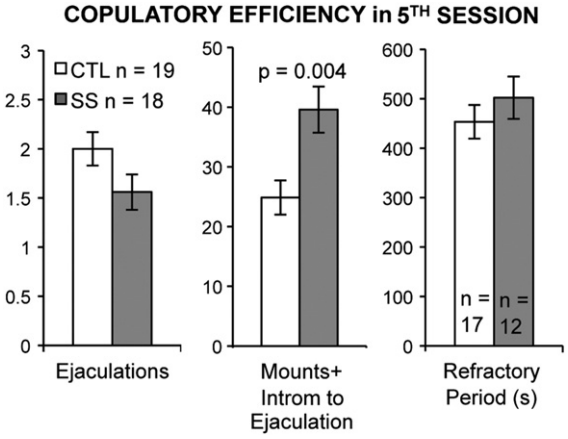

Fig. 3. Mean (±S.E.M.) for the measures of copulatory efficiency for SS and CTL rats.

Testosterone

Testosterone measures from both before the first and after the last sexual behavior test sessions were available for 14 CTL and 16 SS rats. There was no effect of sampling time ($F_{1,28}=1.38$, $p=0.25$, partial $\eta^2=0.05$). CTL rats had higher plasma testosterone concentrations than SS rats ($F_{1,28}=4.84$, $p=0.04$, partial $\eta^2=0.15$), and there was no interaction of Group and Time ($F_{1,28}=0.96$, $p=0.78$, partial $\eta^2=0.003$) (Fig. 6). Testosterone concentrations after the last session were not correlated with behavioral measures for either CTL or SS rats.

Immunohistochemistry

There were no group differences in the number of Fos-ir ($t_{37}=0.95$, $p=0.35$, Cohen's $d=0.31$), AR-ir ($t_{39}=0.52$, $p=0.61$, Cohen's $d=0.17$), or ERα-ir cells ($t_{33}=1.10$, $p=0.59$, Cohen's $d=0.38$) in the mPOA (Fig. 7). Because of the role of the mPOA in sexual performance, we tested the association between number of Fos-ir cells and number of intromissions in the last test session. Fos-ir was correlated with number of intromissions ($r_{39}=0.34$, $p=0.03$), but the correlation was driven by the scores for the SS rats ($r_{19}=.45$, $p=0.05$); the correlation was not significant for the CTL rats ($r_{19}=0.15$, $p=0.53$) (Fig. 7).

Discussion

When tested as adults six weeks after the last stress exposure, rats exposed to social instability stress (SS) in adolescence had deficits in

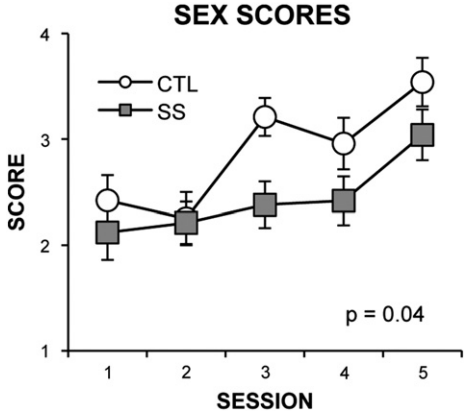

Fig. 4. Mean (±S.E.M.) Sex Score assigned to the sexual performance of each rat by test session (range of 0=no mounts, intromissions, or ejaculation; to 5=three ejaculations).

Please cite this article as: McCormick, C.M., et al., Deficits in male sexual behavior in adulthood after social instability stress in adolescence in rats, Horm. Behav. (2012), http://dx.doi.org/10.1016/j.yhbeh.2012.11.009

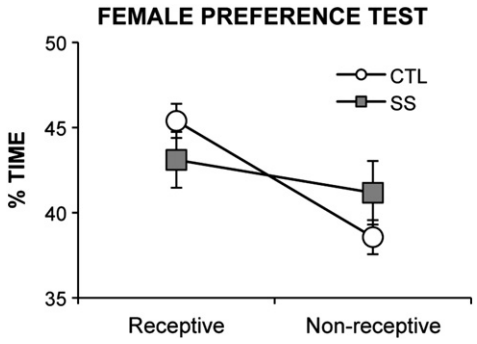

Fig. 5. Mean (±S.E.M.) percent of time that SS and CTL rats spent near a receptive or a non-receptive female during the partner preference test.

sexual performance compared to control rats. Both groups of rats improved performance across test sessions, as indicated by reduced latencies to display, and increased numbers of, mounts, intromissions, and ejaculations from session to session. CTL rats, however, made more ejaculations than did SS rats across sessions. Further, the reduced performance of SS rats was evident in the fifth test session even when only rats that completed an ejaculatory sequence were considered; SS rats displayed more mounts and intromissions before ejaculation, and fewer SS than CTL rats made more than one ejaculation in the last session. Thus, social instability stress in adolescence leads to an enduring deficit in sexual performance. SS and CTL rats did not differ in sexual motivation as gauged by the partner preference test, a test for which preference for an estrous versus a non-estrous female has been a successful gauge of sexual motivation (e.g., Harding and Velotta, 2011; Kelliher and Baum, 2001; Vagell and McGinnis, 1997). Our version of the test, however, may not have been a sensitive enough measure of sexual motivation as the overall preference for a receptive female over a non-receptive female was not statistically significant.

Testosterone and its aromatization to estradiol are necessary for a complete presentation of male sexual behavior (reviewed in Baum, 2003). For example, castrated rats given low testosterone replacement had fewer ejaculations, longer latencies to ejaculation, and a reduced preference for a receptive female in a partner preference test than rats given a high testosterone replacement intended to mimic physiological concentrations (Harding and Velotta, 2011). SS rats had lower plasma concentrations of testosterone than did control rats, but testosterone concentrations did not account for individual differences in sexual behavior within groups. Further, SS and control rats did not differ in counts of cells immunoreactive for either AR or ERα in the mPOA. Others have reported that rats determined to be non-copulators had lower ERα immunoreactive cell counts in the mPOA than did copulators (Portillo et al., 2006), but at most three of the rats in the present study could be considered non-copulators.

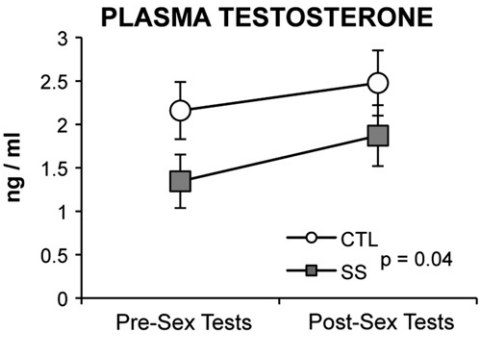

Fig. 6. Mean (±S.E.M.) plasma testosterone concentrations collected before and after sexual behavior testing.

Rather, in many respects, the sexual performance of SS rats fits the definition of "sexually sluggish", a term that refers to animals that display few ejaculations in sexual test sessions. Further, although neither plasma testosterone nor estradiol concentrations distinguished between rats with and without impaired sexual performance, aromatase (responsible for the conversion of testosterone to estradiol) concentrations were lower in the mPOA in "sexually sluggish" rats (Portillo et al., 2007). Thus, reduced testosterone for conversion to estradiol may be a basis for the sexual deficits observed here. Several neurotransmitter systems, and notably dopamine, have been implicated in sexual sluggishness in rats (reviewed in Pfaus, 2009), but its basis continues to be poorly understood (e.g., Antonio-Cabrera and Paredes, 2012).

Another possibility is that changes in the brain and its development at the time of stress exposures may underlie the performance differences of SS rats compared to control rats. Dopamine within the mPOA and released from projections from the ventral tegmental area to limbic and cortical regions facilitates male sexual behavior (reviewed in Hull and Dominguez, 2007), and the dopaminergic system continues to develop in adolescence (Andersen et al., 2000, 2002; Mathews and McCormick, 2012; Mathews et al., 2011). We have some evidence that social instability stress may alter mesocorticolimbic dopamine function based on behavioral differences in response to psychostimulants in SS compared to CTL rats (McCormick, 2010). In addition, SS rats have enduring deficits in hippocampal-dependent memory, altered hippocampal neurogenesis, and changes in proteins associated with synaptic plasticity (McCormick et al., 2012; Morrissey et al., 2011). These results suggest social instability stress alters ongoing brain development, and to the extent that the neural circuitry of sexual behavior continues to mature in adolescence (e.g., Ahmed et al., 2008; De Lorme et al., 2012), it too may be altered by the stress exposures.

We did not find a difference in the number of Fos immunoreactive cells in the mPOA of SS rats compared to control rats after the fifth sexual behavior test session. Our results of performance differences between SS and control rats in the absence of group differences in Fos expression contrast the results found for rats reared in isolation in early life (neonatal) compared to controls whereby groups differences were found in Fos immunoreactive cell counts but not in sexual performance (Akbari et al., 2008). Group differences in sexual behavior in that study, however, may have emerged with more experience; the study involved only one test session. Prenatally-stressed males, which show deficits in sexual behavior as adults (e.g., Wang et al., 2006; Ward, 1972; Ward and Reed, 1985), had longer latencies to mount and decreased Fos expression in the mPOA after the mount compared to control males (Humm et al., 1995). These studies highlight that early life stressors can have a lasting impact on reproductive function. Previous research has found that Fos immunoreactive cell counts are proportional to the amount of sexual activity (Baum and Everitt, 1992). We found a modest association between number of intromissions and Fos cell counts, an association that was significant only within the SS rats. Thus, more detailed analyses of a broader range of regions involved in sexual behavior within and beyond the mPOA may reveal differences between SS and CTL rats.

Another possibility is that the deficits in sexual behavior of SS rats may be secondary to other performance-related factors. Social instability stress in adolescence heightens avoidant (anxiety) behavior without affecting approach (exploration) behavior; SS rats exhibited more anxiety-like behavior in the elevated plus maze and open field tests and reduced interactions in the social interaction test, but did not differ in social approach (Green et al., in press; McCormick et al., 2008). Thus, stress in adolescence may affect later sexual behavior by increasing anxiety and/or preventing the acquisition of an appropriate social repertoire.

A remaining question is whether the effects of the stress exposures on reproductive behavior are unique to adolescence and different from exposures in adulthood. Chronic stress is known to impair

Please cite this article as: McCormick, C.M., et al., Deficits in male sexual behavior in adulthood after social instability stress in adolescence in rats, Horm. Behav. (2012), http://dx.doi.org/10.1016/j.yhbeh.2012.11.009

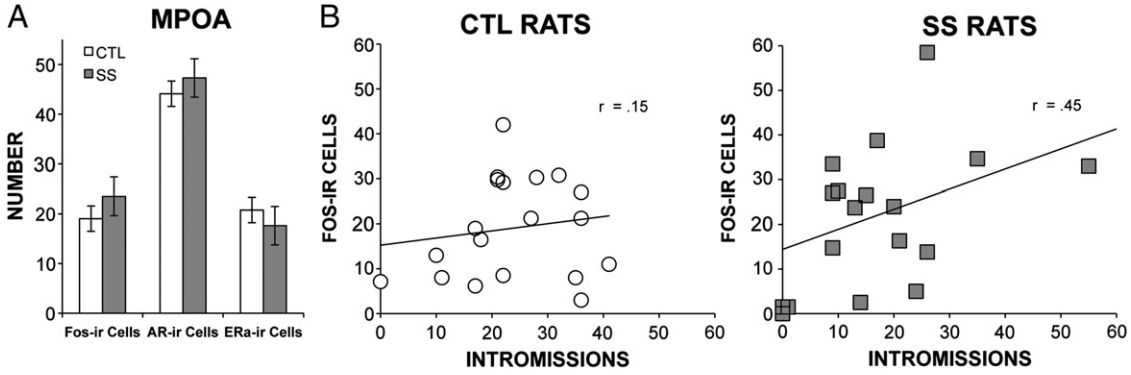

Fig. 7. A. Mean (±S.E.M.) number of cells expressing Fos, AR, or ERα in the medial preoptic area in SS and CTL rats. B. Scatterplots for CTL and SS rats between the number of Fos immunoreactive cells and the number of intromissions in session 5.

sexual performance when experienced in adulthood (e.g., D'Aquila et al., 1994; Gronli et al., 2005; Niikura et al., 2002; Retana-Marquez et al., 2003), although not always (Albonetti and Farabollini, 1993; Gorzalka et al., 1998). In such studies, however, sexual behavior testing was conducted within a short time frame of the stress exposures. Thus, the effects of chronic stress in adulthood on sexual behavior may be short-lived (although one study found deficits in sexual behavior 7 days after a single traumatic stressor experience when reminders of the stress experience were provided (Hawley et al., 2011)), as many of the effects of chronic stress in adulthood on other behavioral and neural measures dissipate with time (e.g., Bian et al., 2012; Dagyte et al., 2009; Luine et al., 1994; Sousa et al., 2000). In contrast to in adulthood, the effects of chronic stressors in adolescence often are observable weeks to months after the stress exposures (reviewed in Green and McCormick, in press), suggesting stress in adolescence alters the trajectory of ongoing brain development. Further, when we compared social instability stress administered in adolescents to social instability stress administered in adults on context conditioning, only adolescent-exposed rats showed deficits when tested within days of, or four weeks after, the stress exposure (Morrissey et al., 2011). A similar psychosocial stress procedure to ours, changes of cage partners for 27 days but administered in adulthood, resulted in no significant difference in sexual behavior compared to controls when tested within a day of the stress exposure (Gorzalka et al., 1998). Combined, these results suggest reproductive function is more susceptible to social stress exposures administered in adolescence than in adulthood. Thus, stressors in adolescence may have "programming" effects on sexual behavior comparable to experience-induced programming effects found at earlier stages of ontogeny (Cameron et al., 2008).

Acknowledgments

The research was funded by the Natural Sciences and Engineering Research Council (NSERC) of Canada and the Canadian Foundation for Innovation (CFI) (PI: CMM). NSERC and CFI had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. We thank Chris Luckart, Jonathan Simone, Jenn McPherson, and Queenie Li for assistance in data collection.

References

Ahmed, E.I., Zehr, J.L., Schulz, K.M., Lorenz, B.H., DonCarlos, L.L., Sisk, C.L., 2008. Pubertal hormones modulate the addition of new cells to sexually dimorphic brain regions. Nat. Neurosci. 11, 995–997.

Akbari, A.M., Budin, R., Parada, M., Fleming, A.S., 2008. The effects of early isolation on sexual behavior and c-fos expression in naive male Long-Evans rats. Dev. Psychobiol. 50, 298–306.

Albonetti, M.E., Farabollini, F., 1993. Effects of single and repeated restraint on the social behavior of male rats. Physiol. Behav. 53, 937–942.

Almeida, S.A., Kempinas, W.G., Lamano Carvalho, T.L., 2000a. Sexual behavior and fertility of male rats submitted to prolonged immobilization-induced stress. Braz. J. Med. Biol. Res. 33, 1105–1109.

Almeida, S.A., Petenusci, S.O., Franci, J.A., Rosa e Silva, A.A., Carvalho, T.L., 2000b. Chronic immobilization-induced stress increases plasma testosterone and delays testicular maturation in pubertal rats. Andrologia 32, 7–11.

Andersen, S.L., Thompson, A.T., Rutstein, M., Hostetter, J.C., Teicher, M.H., 2000. Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. Synapse 37, 167–169.

Andersen, S.L., Thompson, A.P., Krenzel, E., Teicher, M.H., 2002. Pubertal changes in gonadal hormones do not underlie adolescent dopamine receptor overproduction. Psychoneuroendocrinology 27, 683–691.

Antonio-Cabrera, E., Paredes, R.G., 2012. Effects of chronic estradiol or testosterone treatment upon sexual behavior in sexually sluggish male rats. Pharmacol. Biochem. Behav. 101, 336–341.

Baum, M.J., 2003. Activational and organizational effects of estradiol on male behavioral neuroendocrine function. Scand. J. Psychol. 44, 213–220.

Baum, M.J., Everitt, B.J., 1992. Increased expression of c-fos in the medial preoptic area after mating in male rats: role of afferent inputs from the medial amygdala and midbrain central tegmental field. Neuroscience 3, 627–646.

Bian, Y., Pan, Z., Hou, Z., Huang, C., Li, W., Zhao, B., 2012. Learning, memory, and glial cell changes following recovery from chronic unpredictable stress. Brain Res. Bull. 88, 471–476.

Brenhouse, H.C., Andersen, S.L., 2011. Developmental trajectories during adolescence in males and females: a cross-species understanding of underlying brain changes. Neurosci. Biobehav. Rev. 35, 1687–1703.

Brotto, L.A., Gorzalka, B.B., Hanson, L.A., 1998. Effects of housing conditions and 5-HT2A activation on male rat sexual behavior. Physiol. Behav. 63, 475–479.

Bulygina, V.V., Amstislavskaia, T.G., Maslova, L.N., 2005. The effect of social isolation during juvenile period on the sexual arousal in adult rats. Ross. Fiziol. Zh. Im. I M Sechenova 91, 1356–1365.

Cameron, N.M., Shahrokh, D., Del Corpo, A., Dhir, S.K., Szyf, M., Champagne, F.A., Meaney, M.J., 2008. Epigenetic programming of phenotypic variations in reproductive strategies in the rat through maternal care. J. Neuroendocrinol. 20, 795–801.

Coolen, L.M., 2005. Neural control of ejaculation. J. Comp. Neurol. 493, 39–45.

Crews, F., He, J., Hodge, C., 2007. Adolescent cortical development: a critical period of vulnerability for addiction. Pharmacol. Biochem. Behav. 86, 189–199.

Dagyte, G., Van der Zee, E., Postema, F., Luiten, P.G., Den Boer, J.A., Trentani, A., Meerlo, P., 2009. Chronic but not acute footshock stress leads to temporary suppression of cell proliferation in rat hippocampus. Neuroscience 162, 904–913.

D'Aquila, P.S., Brain, P., Willner, P., 1994. Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression. Physiol. Behav. 56, 861–867.

De Lorme, K.C., Schulz, K.M., Salas-Ramirez, K.Y., Sisk, C.L., 2012. Pubertal testosterone organizes regional volume and neuronal number within the medial amygdala of adult male Syrian hamsters. Brain Res. 1460, 33–40.

Doremus-Fitzwater, T.L., Varlinskaya, E.L., Spear, L.P., 2010. Motivational systems in adolescence: possible implications for age differences in substance abuse and other risk-taking behaviors. Brain Cogn. 72, 114–123.

Dorn, L.D., Chrousos, G.P., 1997. The neurobiology of stress: understanding regulation of affect during female biological transitions. Semin. Reprod. Endocrinol. 15, 19–35.

Ferris, C.F., Messenger, T., Sullivan, R., 2005. Behavioral and neuroendocrine consequences of social subjugation across adolescence and adulthood. Front. Zool. 2, 7.

Frahm, K.A., Lumia, A.R., Fernandez, E., Strong, R., Roberts, J.L., McGinnis, M.Y., 2011. Effects of anabolic androgenic steroids and social subjugation on behavior and neurochemistry in male rats. Pharmacol. Biochem. Behav. 97, 416–422.

Gorzalka, B.B., Hanson, L.A., Brotto, L.A., 1998. Chronic stress effects on sexual behavior in male and female rats: mediation by 5-HT2A receptors. Pharmacol. Biochem. Behav. 61, 405–412.

Green, M. R., McCormick, C. M., in press. Effects of stressors in adolescence on learning and memory in rodent models. Horm. Behav.
Green, M. R., Barnes, B., McCormick, C. M., in press. Social instability stress in adolescence increases anxiety and reduces social interactions in adulthood in male Long Evans rats. Dev. Psychobiol. http://dx.doi.org/10.1002/dev.21077 [Sep 21, Epub ahead of print].
Gronli, J., Murison, R., Fiske, E., Bjorvattn, B., Sorenson, E., Portas, C.M., Ursin, R., 2005. Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. Physiol. Behav. 84, 571–577.
Harding, S.M., Velotta, J.P., 2011. Comparing the relative amount of testosterone required to restore sexual arousal, motivation, and performance in male rats. Horm. Behav. 59, 666–673.
Harris, A., Seckl, J., 2011. Glucocorticoids, prenatal stress and the programming of disease. Horm. Behav. 59, 279–289.
Hawley, W., Grissom, E., Keskitalo, L., Hastings, T., Dohanich, G., 2011. Sexual motivation and anxiety-like behaviors of male rats after exposure to a trauma followed by situational reminders. Physiol. Behav. 102, 181–187.
Hebbard, P.C., King, R.R., Malsbury, C.W., Harley, C.W., 2003. Two organizational effects of pubertal testosterone in male rats: transient social memory and a shift away from long-term potentiation following a tetanus in hippocampal CA1. Exp. Neurol. 182, 470–475.
Hull, E.M., Dominguez, J.M., 2007. Sexual behavior in male rodents. Horm. Behav. 52, 45–55.
Humm, J.L., Lambert, K.G., Kinsley, C.H., 1995. Paucity of c-fos expression in the medial preoptic area of prenatally stressed male rats following exposure to sexually receptive females. Brain Res. Bull. 37, 363–368.
Kelliher, K.R., Baum, M.J., 2001. Nares occlusion eliminates heterosexual partner selection without disrupting coitus in ferrets of both sexes. J. Neurosci. 21, 5832–5840.
Llorente, R., Miguel-Blanco, C., Aisa, B., Lachize, S., Borcel, E., Meijer, O.C., Ramirez, M.J., de Kloet, E.R., Viveros, M.P., 2011. Long-term sex-dependent psychoneuroendocrine effects of maternal deprivation and juvenile unpredictable stress in rats. J. Neuroendocrinol. 23, 329–344.
Luine, V., Villegas, M., Martinez, C., McEwen, B.S., 1994. Repeated stress causes reversible impairments of spatial memory performance. Brain Res. 639, 167–170.
Mathews, I.Z., McCormick, C.M., 2012. Role of medial prefrontal cortex dopamine in age differences in response to amphetamine in rats: locomotor activity after intra-mPFC injections of dopaminergic ligands. Dev. Neurobiol. 72, 1415–1421.
Mathews, I.Z., Brudzynski, S.M., McCormick, C.M., 2011. Heightened locomotor-activating effects of amphetamine administered into the nucleus accumbens in adolescent rats. Int. J. Dev. Neurosci. 29, 501–507.
McCormick, C.M., 2010. An animal model of social instability stress in adolescence and risk for drugs of abuse. Physiol. Behav. 99, 194–203.
McCormick, C. M., Green, M. R., in press. From the stressed adolescent to the anxious and depressed adult: investigations in rodent models. Neuroscience. http://dx.doi.org/10.1016/j.neuroscience.2012.08.063. [Sep 9, pii: S0306-4522(12)00898-6, Epub ahead of print].
McCormick, C.M., Mathews, I.Z., 2010. Adolescent development, hypothalamic–pituitary–adrenal function, and programming of adult learning and memory. Prog. Neuropsychopharmacol. Biol. Psychiatry 34, 756–765.
McCormick, C.M., Merrick, A., Secen, J., Helmreich, D.L., 2007. Social instability in adolescence alters central and peripheral HPA responses to a repeated homotypic stressor in male and female rats. J. Neuroendocrinol. 19, 116–126.
McCormick, C.M., Smith, C., Mathews, I.Z., 2008. Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. Behav. Brain Res. 187, 228–238.
McCormick, C.M., Mathews, I.Z., Thomas, C., Waters, P., 2010. Investigations of HPA function and the enduring consequences of stressors in adolescence in animal models. Brain Cogn. 72, 73–85.
McCormick, C.M., Thomas, C.M., Sheridan, C.S., Nixon, F., Flynn, J.A., Mathews, I.Z., 2012. Social instability stress in adolescent male rats alters hippocampal neurogenesis and produces a deficit in spatial location memory in adulthood. Hippocampus 22, 1300–1312.
Morrissey, M.D., Mathews, I.Z., McCormick, C.M., 2011. Enduring deficits in contextual and auditory fear conditioning after adolescent, not adult, social instability stress in male rats. Neurobiol. Learn. Mem. 95, 46–56.
Niikura, S., Yokoyama, O., Komatsu, K., Yotsuyanagi, S., Mizuno, T., Namiki, M., 2002. A causative factor of copulatory disorder in rats following social stress. J. Urol. 168, 843–849.
Paxinos, G., Watson, C., 2005. The rat brain in stereotaxic coordinates. Elsevier, Sydney.
Pfaus, J.G., 2009. Pathways of sexual desire. J. Sex. Med. 6, 1506–1533.
Portillo, W., Diaz, N.F., Cabrera, E.A., Fernandez-Guasti, A., Paredes, R.G., 2006. Comparative analysis of immunoreactive cells for androgen receptors and oestrogen receptor α in copulating and non-copulating male rats. J. Neuroendocrinol. 18, 168–176.
Portillo, W., Castillo, C.G., Retana-Márquez, S., Roselli, C.E., Paredes, R.G., 2007. Neuronal activity of aromatase enzyme in non-copulating male rats. J. Neuroendocrinol. 19, 139–141.
Retana-Marquez, S., Bonilla-Jaime, H., Vazquez-Palacios, G., Martinez-Garcia, R., Velazquez-Moctezuma, J., 2003. Changes in masculine sexual behavior, corticosterone and testosterone in response to acute and chronic stress in male rats. Horm. Behav. 44, 327–337.
Romeo, R.D., 2010. Pubertal maturation and programming of hypothalamic–pituitary–adrenal reactivity. Front. Neuroendocrinol. 31, 232–240.
Sakamoto, H., 2012. Brain–spinal cord neural circuits controlling male sexual function and behavior. Neurosci. Res. 72, 103–116.
Schulz, K.M., Richardson, H.N., Zehr, J.L., Osetek, A.J., Menard, T.A., Sisk, C.L., 2004. Gonadal hormones masculinize and defeminize reproductive behaviors during puberty in the male Syrian hamster. Horm. Behav. 45, 242–249.
Seckl, J.R., 2008. Glucocorticoids, developmental 'programming' and the risk of affective dysfunction. Prog. Brain Res. 167, 17–34.
Sousa, N., Lukoyanov, N.V., Madeira, M.D., Almeida, O.F.X., Paula-Barbosa, M.M., 2000. Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. Neuroscience 97, 253–266.
Swanson, H.H., van de Poll, N.E., 1983. Effects of an isolated or enriched environment after handling on sexual maturation and behaviour in male and female rats. J. Reprod. Fertil. 69, 165–171.
Vagell, M.E., McGinnis, M.Y., 1997. The role of aromatization in the restoration of male rat reproductive behavior. J. Neuroendocrinol. 9, 415–421.
Walker, D.M., Junger, T.E., Gore, A.C., 2009. Developmental profiles of neuroendocrine gene expression in the preoptic area of male rats. Endocrinology 150, 2308–2316.
Wang, C.T., Shuia, H.A., Huang, T.L., Tai, M.Y., Peng, M.T., Tsai, Y.F., 2006. Sexual motivation is demasculinized, but not feminized, in prenatally stressed male rats. Neuroscience 138, 357–364.
Ward, I.L., 1972. Prenatal stress feminizes and demasculinizes the behavior of males. Science 175, 82–84.
Ward, I.L., Reed, J., 1985. Prenatal stress and prepuberal social rearing conditions interact to determine sexual behavior in male rats. Behav. Neurosci. 99, 301–309.

Please cite this article as: McCormick, C.M., et al., Deficits in male sexual behavior in adulthood after social instability stress in adolescence in rats, Horm. Behav. (2012), http://dx.doi.org/10.1016/j.yhbeh.2012.11.009