



Sexual experience confers resilience to restraint stress in female rats

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ARTICLE INFO

Keywords:

Sexual motivation
Contact return latency
Sexual experience
Elevated plus maze
Stress response

ABSTRACT

During paced mating, sexually experienced female rats spend more time with the male, return to the male more quickly after intromission, and exhibit shorter interintromission intervals as compared to sexually naïve rats. Factors that trigger the shift in paced mating behavior are unknown. The present study used the elevated plus maze to test whether anxiety-like behavior differs as a function of sexual experience. Ovariectomized, Long-Evans female rats were primed with estradiol benzoate plus progesterone (EB + P) and then either received four, twice weekly, paced mating treatments to gain sexual experience (Experienced) or remained sexually naïve (Naïve) but were exposed to an empty mating apparatus. In Experiment 1, anxiety-like behavior was compared between Experienced or Naïve female rats that were primed with either EB + P or oil. Significantly more time was spent in open arms under EB + P vs. oil, independent of sexual history. To test whether exposure to an acute stressor before elevated plus maze testing affected anxiety-like behavior, EB + P treated, Experienced or Naïve rats received paced mating (Experiment 2) or restraint (Experiment 3) immediately prior to the elevated plus maze task. Restraint, but not mating, led to less anxiety-like behaviors for Experienced rats compared to Naïve rats. Collectively, our data shows that one component of the shift in paced mating behavior that occurs with sexual experience appears to be altered stress responsiveness. We propose that mating is a beneficial stressor that, when repeated, increases the ability to cope with anxiety-producing events such as aversive components of mating or non-voluntary stressors.

1. Introduction

During a mating encounter, female rats control the receipt of sexual stimulations through paced mating behavior, approaching and retreating from the male rat due to the combined effects of motivation and genitosensory input (Erskine, 1989, 1992). Approach behaviors indicate appetitive aspects of mating (Guarraci and Meerts, 2017; Komisaruk and Whipple, 2000; Paredes, 2009) whereas retreat from the male is usually thought to reflect temporarily aversive aspects of mating (Broekman et al., 1988; Peirce and Nuttall, 1961). Paced mating behavior changes with repeated mating such that sexually experienced female rats spend more time with the male, return to the male more quickly after intromission, and receive intromissions at shorter intervals compared to rats mating for the first time (Jones and Pfau, 2014; Lovell et al., 2007; Meerts et al., 2016; Meerts et al., 2014; Meerts et al., 2015). Additionally, during a sexual preference test, rats that are ovariectomized and injected only with oil spend more time with the male if they are sexually experienced than if they are naïve (Meerts et al., 2016; Meerts et al., 2017). The shift in paced mating behavior suggests that with sexual experience, potentially aversive components of mating that lead to withdrawal diminish over multiple sexual

encounters.

One aspect of copulation that may abate with experience is activation of the stress response system. Sexual stimulation activates both the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) in female rats, leading to increases in corticosterone (Frye et al., 1996) heart rate and blood pressure (Gómez et al., 1996; Terada et al., 2003), and pupil dilation (Szechtman et al., 1985) – at least in sexually naïve rats, although no studies have tested the role of sexual experience. Naïve female rats take longer to approach the male during paced mating, potentially because the male is a source of stress (Meerts et al., 2016). Additionally, female rat mating behavior is sensitive to stimulation of the stress response system. Restraint immediately before mating leads to less time spent with the male rat, fewer mounts compared to unrestrained rats, and reduced receptivity (Uphouse et al., 2014; Uphouse et al., 2005; White and Uphouse, 2004). However, effects of stress on mating behavior may be limited to acute stressors because chronic psychosocial stress enhances lordosis and solicitation behaviors in female rats compared to a no stress condition (Gorzalka et al., 1998; Williams et al., 1992). Such an effect of chronic stress may be similar to the well-recognized attenuation of the physiological stress response by repeated, voluntary activities that elicit

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<https://doi.org/10.1016/j.yhbeh.2018.12.003>

Received 4 September 2018; Received in revised form 13 November 2018; Accepted 4 December 2018

Available online 14 December 2018

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HPA and ANS activation, like exercise and skydiving (Masini et al., 2011; Meyer et al., 2015; Rivera-Brown and Frontera, 2012). Repeated engagement in mating-induced stress may similarly lead to physiological adaptation to stressors in female rats, contributing to the effect of sexual experience on the display of paced mating behavior.

In male rats, some studies report that mating stimulates higher adrenal and gonadal hormone levels and reduced anxiety-like behavior in sexually experienced relative to naïve rats (Bonilla-Jaime et al., 2006; Edinger and Frye, 2007; Meraz-Medina et al., 2017), whereas other studies show no difference based on sexual history (Leuner et al., 2010). In female rats, ovarian hormones, which are elevated in a sexually receptive state, decrease anxiety-like behavior (Frye et al., 2000; Marcondes et al., 2001; Nyuyki et al., 2011). Sexually naïve, nulliparous female rats exhibit more anxiety-like behavior than female rats with both mating and maternal experience independent of a proximate mating encounter (Byrnes and Bridges, 2006; Rima et al., 2009). Whether mating, without maternal experience, affects anxiety-like behavior in female rats is unknown.

Collectively, the change in paced mating behavior following sexual experience and data linking mating and stress led to the hypothesis that differences in paced mating behavior may be due to adaptation of the stress response, evident in lower levels of anxiety-like behavior in sexually experienced relative to naïve rats. The elevated plus maze is frequently used to assess the effects of stressors on anxiety-like behavior; time spent in the open arms of the plus maze is considered inversely related to anxiety (Walf and Frye, 2007). We used the elevated plus maze to assess whether hormone priming and sexual experience interact to affect anxiety-like behavior (Experiment 1), and whether sexually experienced and naïve rats differ in anxiety-like behavior immediately after mating (Experiment 2) or immediately after restraint stress (Experiment 3).

2. Materials and methods

2.1. Subjects

86 adult female Long-Evans rats weighing approximately 200 g obtained from Harlan Laboratories (Indianapolis, IN) were used in this experiment. Rats were pair-housed in polycarbonate cages (23 cm W × 43 cm L × 18 cm H) in a temperature and light controlled vivarium (12:12-h light/dark cycle, lights off at 1100 h), with commercial rat pellets and water available ad libitum. All female rats were ovariectomized under ketamine (50 mg/kg) xylazine (2.5 mg/kg) anesthesia 7–10 days prior to the start of behavioral testing. All behavioral testing occurred during the dark period of the light cycle under dim red light. To induce receptivity, female rats received subcutaneous injections of 10 µg estradiol benzoate (EB, Sigma-Aldrich, St. Louis, MO) 48 h and 1 mg progesterone (P, Sigma) 4 h prior to testing (EB + P). Hormones were dissolved in reagent grade sesame oil vehicle (Sigma). Sexually experienced, adult male Long-Evans rats were used as stimulus rats. The Institutional Animal Care and Use Committee at Carleton College approved all procedures in accordance with NIH guidelines.

2.2. Apparatus and procedures

2.2.1. Exposure and mating

Exposure or mating occurred in a Plexiglas arena (112.5 cm W × 37.5 L × 32 cm H) that could be partitioned into three equal compartments using two pairs of Plexiglas dividers (a clear divider with 5-cm holes in the bottom corners, and an opaque, solid divider). Aspen bedding covered the floor of the chamber. Rats were familiarized to an empty arena for two, separate 15 min periods the week before beginning behavioral testing.

Rats assigned to remain sexually naïve were hormone primed and then received four, twice weekly, exposure treatments. Rats were placed in the center compartment of the arena for a 5-min acclimation

period before one opaque divider was raised, affording the rat access to the center compartment and an empty outer compartment for 15 min. Rats assigned to receive sexual experience were hormone primed and then received four, twice weekly, paced mating treatments on the same days and in the same room as the naïve rats that received exposure treatments. The female rat acclimated in the center compartment for 5 min, while a sexually active male rat acclimated in each outer compartment. The test began when the experimenter raised one of the two opaque dividers, granting the female access to one male rat and continued until the female rat received 15 intromissions including ejaculations. If the first male rat ejaculated before the 15th intromission, the experimenter waited for the female rat to exit and then return to the male compartment, at which point the timer was paused, the female rat was returned to the center compartment, and the test resumed with the second male (Meerts et al., 2016). During a test of paced mating, experimenters recorded the time of entries and exits to and from the male compartment, along with the timing and type of sexual stimulation (i.e., mounts, intromissions, ejaculations) and the accompanying lordosis response (Hardy and DeBold, 1972). Number of proceptive (e.g., hop/darts and ear wiggles) and rejection (kicks, squeaks, rolls) behaviors (Erskine, 1989; Madlafousek and Hlinak, 1977; Pfaus et al., 2003) and length of test were also recorded. Paced mating behaviors were calculated from these measures: (a) contact-return latency, the elapsed time between receipt of a sexual stimulation and return to the male after exiting the male compartment; (b) percentage of exits following stimulation, number of times the female exited the male compartment after receiving a sexual stimulation × 100; (c) percent of the test spent in the male compartment; (d) interintromission interval, the test duration divided by number of intromissions plus ejaculations; and (e) latency to first enter the male compartment.

2.2.2. Elevated plus maze

The elevated plus maze was made of black Plexiglas and consisted of two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm × 40 cm high) arranged in a plus shape. The maze was elevated 50 cm above the ground. Illumination was 20 lux in closed arms and 140 lux in open arms (Nyuyki et al., 2011; Walf and Frye, 2007). The test began when a rat was placed in the center of the maze, facing the open arm away from the experimenter. The experimenter counted the number of entries onto the open arms and used a stopwatch to measure time in the open arms. An open arm entry was counted when all four paws crossed into the open arm. If a rat fell off of the maze, the experimenter would gently place the rat back on at the same place and orientation. At the conclusion of the 5-min test, the rat was returned to the home cage, and the maze was thoroughly cleaned with 70% alcohol and dried with paper towels before the next test. Plus maze tests for each experiment were carried out by a single experimenter, who was unaware of the condition to which each rat was assigned.

2.2.3. Restraint stress

Disposable DecapiCones™ were used for restraint to induce a stress response. Rats were placed head first into the DecapiCone™, which is a tapered plastic tube with a small opening at the tip of the bag. Once the rat was in the tube, the base of the cone was gathered and secured with tape to prevent escape. Rats were placed in the tube for 10 min, which induces elevated corticosterone levels (Gadek-Michalska and Bugajski, 2003). The no restraint control condition consisted of placing the rat alone in a small (39.4 × 22.9 × 31.1 cm high) holding chamber for 10 min.

2.3. Experimental design

Experiment 1 was conducted to test the hypothesis that anxiety-like behavior would differ as a consequence of sexual history in rats when injected with EB + P but not when injected with oil. Rats were randomly assigned to remain sexually naïve (Naïve, $n = 10$) or to become

sexually experienced (Experienced, $n = 12$), as described above. The week following four exposure treatments (Naïve) or four paced mating treatments (Experienced), all rats were injected with the sesame oil vehicle 48 h and 4 h prior to elevated plus maze testing. On a different day, rats were tested on the elevated plus maze 48 h after EB and 4 h after P. Rats were placed onto the elevated plus maze directly from the home cage.

Experiment 2 was conducted to test the hypothesis that anxiety-like behavior would be higher in naïve than experienced female rats if a mating encounter immediately preceded the elevated plus maze test. Rats were randomly assigned to three groups: Naïve/Mated, $n = 12$; Experienced/Mated, $n = 14$; Naïve/Exposure, $n = 11$. Exposure and paced mating treatments occurred as described above. Naïve/Mated rats were tested on the elevated plus maze immediately after their first paced mating encounter, Experienced/Mated rats were tested on the elevated plus maze immediately after their fifth paced mating encounter, and Naïve/Exposure rats were tested on the elevated plus maze immediately after exposure treatment.

Experiment 3 was conducted to test the hypothesis that elevated plus maze behavior would differ between hormone-primed, experienced and naïve rats after restraint stress but not in the absence of restraint. Restraint, a non-voluntary stressor, was chosen because it is known to increase stress hormones (Kalil et al., 2013; Kudwa et al., 2014) and modulate anxiety-like behavior (Jones et al., 2016). Rats that had received five mating encounters or exposures to the empty paced mating chamber were randomly assigned to receive restraint or no restraint, leading to four groups: Experienced/Restraint, $n = 8$; Experienced/No Restraint, $n = 5$; Naïve/Restraint, $n = 9$; Naïve/No Restraint, $n = 5$. Rats received EB 48 h and P 4 h prior to testing and were placed onto the elevated plus maze immediately after the 10-min restraint stress or no restraint (control) treatment.

2.4. Data analysis

Paired t -tests were used to verify that measures of paced mating behavior changed in the sexually experienced groups from the first mating test to the fourth mating test. Analysis of variance (ANOVA) was used to assess effects of manipulations on time in open arms and number of open arm entries. Data from Experiment 1 were analyzed with a 2 (hormone: EB + P or oil) \times 2 (sexual history: Experienced or Naïve) ANOVA with repeated measures on hormone. Data from Experiment 2 were analyzed with a one-way ANOVA and data from Experiment 3 were analyzed with a 2 (condition: Restraint or Control) \times 2 (sexual history: Experienced or Naïve) ANOVA. Due to our a priori hypothesis that plus maze behavior would differ between Experienced and Naïve rats in the restraint but not the control condition, a planned contrast was conducted to separately compare plus maze behavior between Experienced and Naïve rats in each condition. Effect sizes were estimated using Cohen's d for t -tests and eta squared for ANOVAs. The alpha level was set at $p < 0.05$.

3. Results

3.1. Experiment 1

As expected, on the fourth test of paced mating behavior, sexually experienced rats returned to the male significantly more quickly after intromissions ($t(9) = 3.7$, $d = 1.4$), had significantly shorter inter-intromission intervals ($t(9) = 3.8$, $d = 1.3$), spent significantly more time with the male ($t(9) = 3.8$, $d = 1.2$), and exhibited a significantly shorter latency to enter the male compartment initially ($t(9) = 3.7$, $d = 1.4$) than on the first (naïve) test of paced mating behavior (Table 1). Independent of sexual history, female rats spent significantly more time on the open arms and made more entries into the open arms of the plus maze under EB + P than under oil (Fig. 1). The ANOVA revealed a significant main effect of hormone treatment for time in

open arms ($F(1, 20) = 7.74$, $\eta_p^2 = 0.28$) and entries into open arms ($F(1, 20) = 42.2$, $\eta_p^2 = 0.68$) but no main effect of sexual history and no interaction.

3.2. Experiment 2

Paced mating behavior changed significantly as found in Experiment 1, (all t 's > 2.3 , d 's ranged from 0.7 to 1.3; data not shown). No significant differences in time in open arms or entries into open arms were found between the three groups (Fig. 2).

3.3. Experiment 3

Paced mating behavior changed significantly as found in Experiment 1 and 2, (all t 's > 2.2 , d 's ranged from 0.7 to 1; data not shown). Sexually experienced rats spent more time on the open arms and made more entries into the open arms than sexually naïve rats following restraint whereas no differences were observed between experienced and naïve rats in the no restraint control condition (Fig. 3). No significant differences were found in the omnibus ANOVA, but the planned comparisons showed a significant difference after restraint between experienced and naïve rats with respect to time in open arms ($F(1,15) = 6.22$, $\eta_p^2 = 0.29$) and number of entries into open arms ($F(1,15) = 8.34$, $\eta_p^2 = 0.36$).

4. Discussion

The present study tested the hypothesis that anxiety-like behavior is modified by sexual experience in combination with ovarian hormones and exposure to an acute stressor prior to elevated plus maze testing. In agreement with previous findings, ovarian hormones have anxiolytic properties (Frye et al., 2000; Marcondes et al., 2001; Nyuyki et al., 2011) and sexually experienced female rats show a pattern of paced mating behavior characterized by increased approach toward the sexually active male rat (Table 1; Jones et al., 2013; Lovell et al., 2007; Meerts et al., 2016; Meerts et al., 2014; Meerts et al., 2015). We extend these findings to show that sexual history affects anxiety-like behavior in female rats following restraint stress, but not when tested directly from the home cage or after a mating encounter. Together, the lower levels of withdrawal behaviors during mating and the reduced anxiety-like behavior in experienced relative to naïve rats after restraint suggest that sexual experience produces adaptation of the stress response, thereby buffering against stressors, including mating-induced stress.

In Experiment 1, rats treated with EB + P spent more time in the open arms of the elevated plus maze than rats injected with oil although no effect of sexual history was observed (Fig. 1). Our data agrees with other studies showing that ovarian hormones attenuate anxiety-like behavior (Frye et al., 2000; Nyuyki et al., 2011). Initiating copulation with a larger male rat may be dependent on the anxiolytic effect of ovarian hormones in concert with hormone-based increases in sexual motivation and receptivity. The lack of effect of sexual history on anxiety-like behavior in rats tested directly from the home cage led us to suspect that the difference in paced mating behavior between experienced and naïve female rats was linked to acute stress encountered as part of mating.

Exposure to an acute stressor, including mating, before elevated plus maze testing can alter anxiety-like behavior (Carobrez and Bertoglio, 2005; Nyuyki et al., 2011). Both mating and restraint increase corticosterone and progesterone levels relative to baseline (Frye et al., 1996; Kalil et al., 2013). Therefore, we tested whether receiving mating or restraint immediately prior to elevated plus maze testing altered anxiety-like behavior as a function of sexual history. Restraint immediately prior to elevated plus maze testing led to less time in the open arms in naïve relative to experienced rats (Fig. 3). In contrast, no difference in anxiety-like behavior was observed when mating occurred immediately before elevated plus maze testing (Fig. 2). The discrepant

Table 1
Measures of paced mating behavior.

	Contact-return latency to intromission	Interintromission interval	Percentage of time with male	Latency to enter male compartment
Test 1 (Naïve)	38.9 ± 8.1	77.7 ± 14.6	23.8 ± 4.3	33.7 ± 8.1
Test 4 (Experienced)	9.6 ± 0.7*	18.5 ± 1.4*	47.2 ± 4.8*	2.7 ± 0.7*

Means ± SEM are shown.

* Significantly different from Test 1, $p < 0.05$.

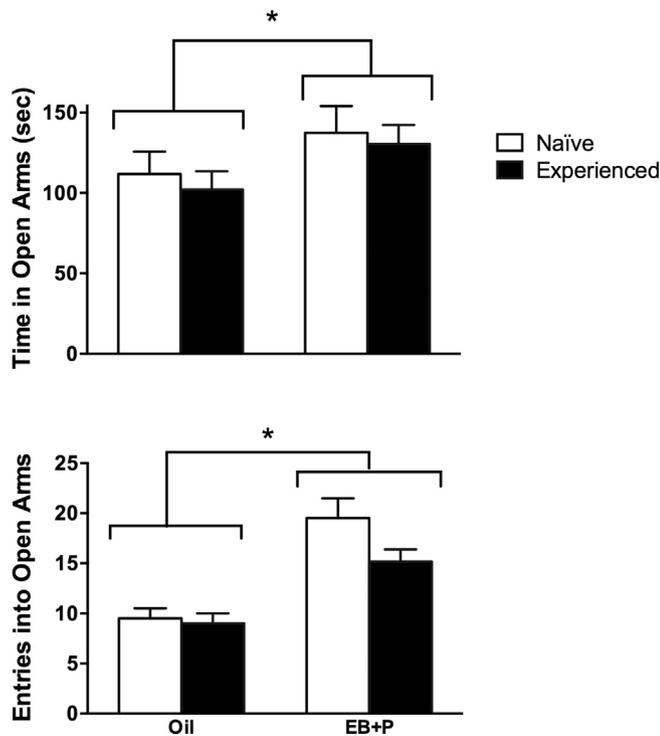


Fig. 1. Time in open arms, as well as entries into open arms, increased as a function of hormone priming, independent of sexual history ($*p < 0.05$). Mean ± SEM seconds spent in open arms and number of entries into the open arms during a 5-min elevated plus maze test is shown for sexually naïve (white bars, $n = 10$) or sexually experienced (black bars, $n = 12$) female rats tested from the home cage. Rats were tested after sesame oil injections (OIL) or EB + P on two separate days.

effect of these two acute stressors is likely related to the specific physiological response provoked by each stressor.

A more robust stress response along with differences in neurobiological substrates appears to alter stress coping behavior. Restraint is a more intense stressor than mating, illustrated by the 2–3 fold higher levels of plasma corticosterone and progesterone induced by restraint relative to mating (Frye et al., 1996; Kalil et al., 2013). Additionally, mating before elevated plus maze testing may not affect anxiety-like behavior because the complex nature of a mating interaction includes both appetitive and aversive qualities. Mating stimulates the HPA axis and ANS, but could be considered a pleasurable stressor because female rats voluntarily approach a male rat to receive sexual stimulation and mating is rewarding to female rats (Komisaruk and Whipple, 2000; Meerts and Clark, 2007; Paredes and Vazquez, 1999). The positive affective state induced by mating via the endogenous opioid system lasts for at least 30-min (Garcia-Horsman et al., 2008), and may explain, in part, why mating prior to elevated plus maze testing does not alter anxiety-like behavior. The stressful aspects of mating for the first time (e.g., a novel male conspecific, unfamiliar sensory stimulation) may affect the display of paced mating behavior as it unfolds but once mating ends, the source of stress is removed leaving mainly the positive affective state. Overall, mating appears to engage mechanisms that

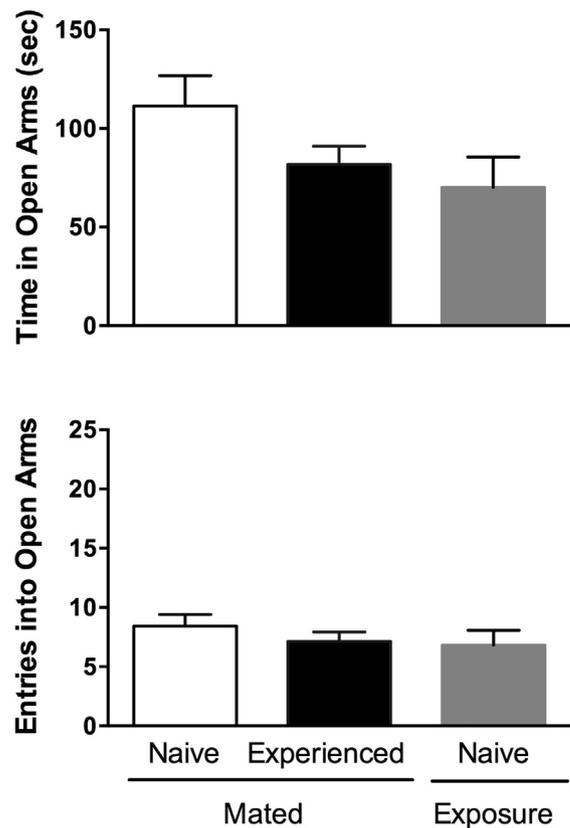


Fig. 2. Groups did not differ significantly as a function of sexual history or event preceding elevated plus maze test. Mean ± SEM seconds spent in open arms and number of entries into the open arms during a 5-min elevated plus maze test is shown. Naïve/Mated (white bars, $n = 12$) rats were tested immediately after their first mating encounter; Experienced/Mated (black bars, $n = 14$) rats were tested immediately after their fifth mating encounter; Naïve/Exposure (gray bars, $n = 11$) rats were tested immediately after 15-min exposure to an empty paced mating chamber.

counteract the negative effects of stress hormones and ANS activation, especially once mating concludes.

The increased time in open arms after restraint stress shown by experienced female rats relative to naïve female rats is consistent with the idea that repeated mating is a beneficial stressor, similar to exercise (Campeau et al., 2010; Jones et al., 2016). The complete physiological response of a mating encounter may consist of mechanisms that protect against the negative effects of elevated levels of corticosterone (Romero et al., 2009; Schoenfeld and Gould, 2012). Exactly what constitutes the physiological response elicited by exposure to stressors in experienced relative to naïve female rats remains to be determined. In addition, the specific aspects of the mating interaction (e.g., social contact or vaginocervical stimulation) that aid in responding to stress is unknown. Estradiol and progesterone attenuate anxiety-like behavior (Fig. 1, Frye et al., 2000; Marcondes et al., 2001; Nyuyki et al., 2011) and are released in response to restraint stress (Hueston and Deak, 2014; Kalil et al., 2013; MacNiven and Younglai, 1992) making differences in either levels of these hormones or receptors good candidates for

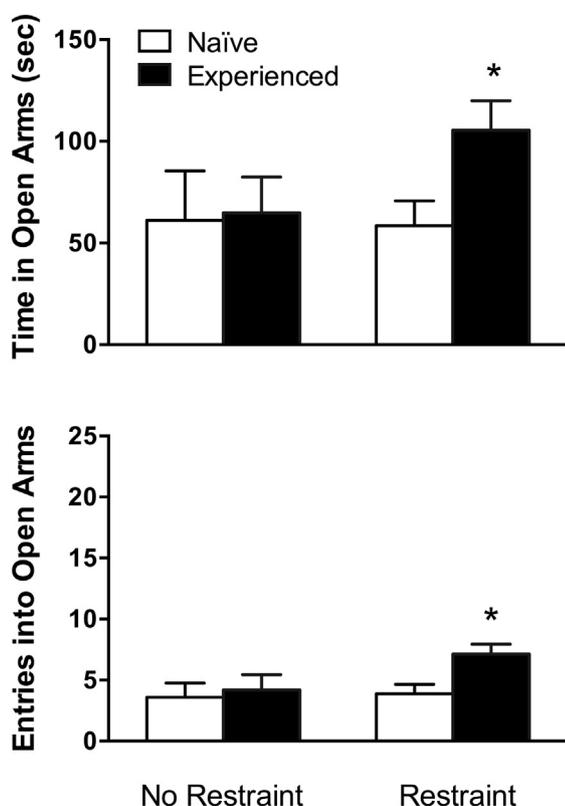


Fig. 3. When tested immediately following 10 min of restraint stress, sexually experienced rats (black bars) spent significantly more time in the open arms and made more entries into open arms relative to sexually naïve rats (white bars; $*p < 0.05$). Mean \pm SEM for seconds spent in open arms and number of entries into the open arms during a 5-min elevated plus maze test immediately following 10 min of restraint stress or exposure control. Rats were tested after injections with EB + P. Data is shown for rats assigned to Experienced/Restrained, $n = 8$; Experienced/No Restraint, $n = 5$; Naïve/Restrained, $n = 9$; Naïve/No Restraint, $n = 5$.

mediating behavioral differences arising from sexual experience. Progesterone release also increases after mating in female rats (Erskine, 1987; Frye et al., 1996), so a common mechanism could explain differences in anxiety-like and paced mating behavior observed in sexually experienced compared to naïve female rats. Further research is needed to determine the physiological effects of sexual experience.

Together with the ovarian hormones that promote interest in a sexually active male rat (Clark et al., 2004; Meerts et al., 2016) and reduce anxiety (Frye et al., 2000; Marcondes et al., 2001), adaptation of the stress response may enhance reproductive success in female rats. The mating pattern exhibited by sexually experienced female rats leads to more stimulations, which is associated with an increased likelihood of pregnancy (Coopersmith and Erskine, 1994). Furthermore, in the wild, rats live in a burrow system that provides female rats with a location to withdraw to when pacing the receipt of mating stimulation, whereas engaging with a sexual partner outside of the burrow occurs under the threat of predators (Calhoun, 1963; McClintock et al., 1982). Accelerated receipt of stimulations, once sexual experience is established, would expose the rat to predation for a shorter period of time, an advantageous mating pattern that would increase both survival and reproduction.

The present study is the first to show that sexual experience in female rats alters the ability to cope with an anxiety-producing event. Our data suggests that one component of the shift in paced mating behavior that occurs with sexual experience appears to be altered stress responsiveness. Additional research is necessary to determine the physiological mechanisms altered by sexual experience that confer

resilience in the face of stress.

Acknowledgements

We are thankful to Sophie Guterl, Sabrina Velasco, Jenny Fehring, Ria Sekhawat, Lauren Azuma, Malavika Suresh, Alanna Bares, Lauren Sompil, Madeline Topf, Jacob Schwartz, Maurice Hicks, Antonia Piergies, and Shannon Branigin for their assistance with behavioral testing, discussions, and feedback on earlier drafts. This study was supported by Carleton College.

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