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FEMALE GONADAL HORMONES, MILD RESTRAINT, AND MALE PREFERENCE

L. Uphouse^{*}, C. Hiegel, J. Sarkar, J. Hurlburt, C. Templeton, J. Guptarak, and N. Maswood
Department of Biology, Texas Woman's University, PO Box 425799, Denton, TX 76204

Abstract

The partner preference paradigm was used to test the hypothesis that mild restraint reduced sexual motivation of female rats. Ovariectomized rats were primed with 10 µg estradiol benzoate or estradiol benzoate and 500 µg progesterone. Additional rats were injected with sesame seed oil. These three groups of rats (oil-oil, estradiol benzoate-oil, or estradiol benzoate-progesterone; OO, EO, EP) were placed for 10 min in an arena, the ends of which enclosed either a sexually active male or an ovariectomized, unprimed female. Time spent near the sexually active male relative to time spent near either stimulus animal was used as the index of male preference. As expected, hormonal treatment significantly increased male preference. After this first 10 min interval, females were returned to the home cage or restrained for 5 min in a Decapicone®. Thereafter, male preference was recorded for another 10 min. Consistent with the first 10 min period, EP rats spent significantly more time near the male than did OO rats while EO rats were intermediate. There was no effect of restraint, but there was a significant increase in self-grooming. These findings contrast with previous studies and allow the suggestion that a brief, mild restraint fails to influence the female's sexual motivation. The implications of these findings are discussed.

Keywords

Stress; hormones; ovariectomized females; grooming; sexual motivation; females

1.0 Introduction

In female rodents, behavioral and physiological events required for successful reproduction are coordinated by sequential hormonal priming with estrogen and progesterone which increases both receptive and proceptive behaviors (Beach, 1976; Pfaff, 1970; Sodersten, 1981). Receptivity is usually measured with the lordosis quotient (frequency with which a female exhibits arching of the back in response to the male's mounts). Estrogen, alone, is sufficient for the elicitation of behavioral receptivity but progesterone facilitates the behavior (Pfaff and Modianos, 1985). Proceptivity includes various solicitous behaviors (hopping and darting) the female exhibits that increase the male's attention to the female and requires progesterone as well as estrogen (Erskine, 1989; Sodersten, 1981). In addition, progesterone enhances aspects of female sexual behavior that may be closely associated with the female's motivation to mate (Clark, et al., 2004; Frye, 2007). While such hormonal priming is essential

*Corresponding author: Department of Biology, Texas Woman's University, Denton, TX 76204, Phone: 940-898-2356, FAX: 940-898-2382, Email: Luphouse@mail.twu.edu.

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for reproductive behavior to occur, in the natural environment, whether mating does or does not occur may also depend on environmental conditions that require the female to choose among competitive behaviors (DeVries, 2002; Uphouse, 2000). In the natural setting, the female emerges from the safety of her burrow to find a potential mate. Consequently, fear or anxiety associated with interaction with a mate or entry into a novel environment must be reduced. Thus, hormonal priming facilitates the emergence of behaviors suitable for successful reproduction and simultaneously reduces sensitivity to stimuli elicited responses that would compete with reproductive behavior (Frye, et al., 2006).

Responses to stress include activation of the hypothalamic-pituitary-adrenal (HPA) axis and elevation of a variety of neuropeptides and secretion of pituitary hormones (Carrasco and Van de Kar, 2003; Herman and Cullinan, 1997; Herman, et al., 2003). An interaction between the hypothalamic-pituitary-adrenal axis (HPA) and the hypothalamic-pituitary-gonadal axis is well established (Dobson, et al., 2003; Rivest and Rivier, 1995; Schiml and Rissman, 2000; Viau, 2002). Gonadal hormones can modify HPA function (Figueiredo, et al., 2002; Haas and George, 1988) and activation of the HPA axis can modify reproduction (Rivest and Rivier, 1995; Schiml and Rissman, 2000). On the longer-term, when chronic exposure to a stressor occurs, the female's estrous cycle may be disrupted which probably accounts for many of the effects on female sexual behavior. However, there are few studies of the effects of an acute stress on female sexual behavior. We recently reported that sexually receptive, estrogen-primed female rats showed a decline in lordosis behavior after 5 min restraint (Truitt, et al., 2003; White and Uphouse, 2004). Mild restraint also reduced the female's emergence from an experimental "burrow" in a paradigm where a sexually receptive female could choose to mate with a sexually active male or escape to an experimental "burrow" (Uphouse, et al., 2005). Whether primed with estrogen and progesterone or only with estrogen, restrained females spent less time with the male and more time in the "burrow" (Uphouse, et al., 2005). While it is possible that the female's sexual motivation was reduced by the restraint, it is also possible that restraint-induced fear or anxiety produced a state that interfered with the female's motivation to mate (e.g. the female's behavior after restraint reflected a compromise between the motivation to mate and the fear or anxiety induced by the restraint) and may not have accurately reflected the effect of restraint on the female's sexual motivation.

Such a competition between behaviors that characterize different motivational states has been previously described. For example, female hamsters, subjected to short-term food restriction (which does not interrupt estrous cyclicity), will chose food in preference to a sexual object (Schneider, et al., 2007). Similarly, depending on the intensity of the stimulus, responding to noxious stimuli can supercede responding for food (LaGraize, et al., 2004). However, the choice animals make among competing behaviors varies depending on the degree to which the intensity of the underlying drive states differ (LaGraize, et al., 2004). Consequently, when two competing drives exist, behavior indicative of only a single motivational state may emerge, but the competing state is still present.

In the following experiment, the partner preference paradigm was used to examine the effect of mild restraint on female sexual motivation. In this procedure, the female was placed in an open area with chambers located at each end; one chamber enclosed a sexually active male and the other enclosed a nonsexual (ovariectomized female) incentive animal. The time females spend in the vicinity of the male is thought to reflect the female's sexual motivation (Clark, et al., 2004; Guarraci and Clark, 2006) and, in ovariectomized females, is increased by estrogen and progesterone (Clark, et al., 2004). In this paradigm, restraint may still precipitate fear and/or anxiety, but it is unlikely to result in a behavioral response that is competitive to spending time with the male. Animals subjected to fearful/stressful stimuli generally spend less time in the center of an open arena (Mashoodh, et al., 2008; Turri, et al., 2001) so that restraint would be expected to reduce time in the open area and increase time near one or both of the incentive

animals. Therefore, the partner preference paradigm may allow a more accurate assessment of the effects of restraint on female sexual motivation without interference from a competitive behavior elicited by the stressor.

2.0 Experimental Procedures

2.1 Materials

Disposable Decapicone® restrainers were purchased from Braintree Scientific Inc. (Braintree, MA). Estradiol benzoate, progesterone, and sesame seed oil were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Isoflurane (Aerrane®) and suture materials were purchased from Henry Schein, Inc. (Mellville, NY). All other supplies came from Fischer Scientific (Houston, TX).

2.2 Animals and housing conditions

Adult female Sasco Fischer rats were purchased from Charles River Laboratories (Wilmington, MA) and housed 3 per cage in polycarbonate cages ($45.72 \times 24.13 \times 21.59$ cm) in rooms maintained at 25° C and 55% humidity and with a 12 hr-12 hr light/dark cycle with lights off at 12 noon. Food and water were available *ad libitum*. All procedures were in accordance with the NIH Guide for the Care and Use of Animals in Research and were approved by the Institutional Animal Care and Use Committee at Texas Woman's University.

2.3 Hormonal treatment

After a two-week adaptation to the facility, rats were anaesthetized with AErrane® and ovariectomized as previously described (White and Uphouse, 2004). Approximately 2 weeks after ovariectomy, rats were injected subcutaneously (s.c.) with 0.1 ml containing 10 µg estradiol benzoate (in sesame seed oil) or the oil vehicle. Forty-eight hr later, rats were injected s.c. with 500 µg progesterone (in sesame seed oil) or the oil vehicle (0.1 ml/rat). This led to three priming conditions: oil-oil (OO), estradiol benzoate-oil (EO), and estradiol benzoate-progesterone (EP). On the day of the first injection and on the following day, rats were individually adapted to the male preference testing apparatus for 30 min/day. Partner preference testing took place during the dark cycle, 4–6 hr after the progesterone or oil injection on the third day.

2.4 Partner preference testing

Partner preference was measured in a clear plexiglass box ($91.44 \times 31.75 \times 31.12$ cm). At the ends of the apparatus were two compartments (each 16.51 cm long) separated from the main area by hardware cloth. An ovariectomized female was housed at one end and a sexually active male was housed at the opposite end. These stimulus animals were placed in the area behind the hardware cloth and were physically inaccessible to the test female. The positioning of the stimulus animals was counterbalanced across tests. Prior to the day's test, the female was allowed to explore the apparatus for 5 min. Stimulus animals were not present. The female was briefly removed while the stimulus animals were placed in their compartment and then replaced in the center of the apparatus.

The compartment accessible to the test female was divided into five distinct areas by markings on the floor of the apparatus. From left to right, these areas measured 12.7, 14.48, 6.35, 14.48, and 12.7 cm. The female was placed in the center (6.35 cm center area) of the apparatus and allowed to move freely throughout the apparatus for 10 min. The test female's behavior was videotaped for later analysis. The female was then removed from the apparatus and either returned to her home cage or restrained (see below) for 5 min. Thereafter, females were placed back in the center of the apparatus and videotape recording continued for an additional 10 min.

When all four of the test female's feet were in one of the lateral most 12.7 cm areas, adjacent to either the stimulus male or stimulus female, the test female was scored as spending time near that stimulus animal. Seconds spent near the male (TWM) or the female (TWF) were summed as the test female's total social time (TST). TWM divided by TST was used as the index of male preference. In addition, the number of times the female crossed the center of the apparatus and the total time the female spent in self-grooming were recorded. A female was scored as crossing the center area when all four feet had traversed the 6.35 cm center area.

Two different individuals scored each videotape. The individual scores were compared by Pearson's rho (correlations ranged from a low of 0.781 for grooming in the first 10 min to a high of 0.962 for male preference in the second 10 min) and data from the two individuals were averaged prior to statistical analysis.

2.5 Restraint procedures

Restraint procedures were as previously described (White and Uphouse, 2004). The female was placed head first into a Decapicone® so that her nose was flush with the small opening at the tip of the cone. The base of the cone was then gathered around the female's tail and secured tightly with tape. With this procedure, the female was tightly wrapped within the cone and was unable to turn. Generally, the process of wrapping the female required between 30 and 60 sec. The female remained for 5 min in the Decapicone®. Control (not restrained) rats were returned to their home cage for a comparable time period.

2.6 Statistical procedures

Data were computed for the first (before differential experience) and second (after either 5 min restraint or home cage experience) 10 min time periods in the testing apparatus. Data collected were the time spent with the male, time spent with the female, number of times the female crossed the center of the apparatus, and time spent in self-grooming. Effects of the three priming treatments were evaluated by one-way ANOVA for the first 10 min in the apparatus. Effects of the restraint or home cage experience were evaluated by two-way repeated measures ANOVA with hormone priming and type of experience as main factors and time as the repeated factor (Zar, 1999). Post-hoc comparisons of individual means were conducted with Tukey's test. Alpha was set at 0.05. Correlations between two scorers were computed using Pearson's procedures. Statistical tests were performed using Superanova (v 1.11) from Abacus Concepts.

3.0 Results

Behavior during the first 10 min, prior to the 5 min restraint or home cage experience, was used to assess the effects of hormone. Two females (EO1 and OO8, both of which were in the restraint group) showed no mobility during the first 10 min period and data for these rats were removed from all analyses. As anticipated from prior findings, hormone treatment significantly altered the females' time spent with the male (Figure 1; $F_{2,45} = 5.15$, $P \leq 0.01$). EP rats spent the most time with the male while OO rats spent significantly less time with the male ($q_{45,3} = 4.36$, $P \leq 0.05$). EO rats were intermediate in time spent with the male and did not differ significantly from either OO or EP rats.

Repeated measures ANOVA with hormone treatment and type of experience (home cage or restraint) as main factors and time as the repeated factor was used to examine the effects of restraint in the 3 priming treatments. As evident from Figure 1, there was not a significant effect of restraint ($F_{1,42} = 0.948$, $P > 0.05$) and no interactions were significant. Across the 20 min test, there continued to be significant effects of hormone treatment on male preference (e.g. TWM/TST) ($F_{2,42} = 6.59$, $P \leq 0.004$). EP rats spent significantly more time with males than did OO ($q_{42,3} = 8.28$) or EO ($q_{42,3} = 6.14$) rats and this pattern was unaffected by the

restraint. Social time (e.g. time spent with either of the incentive animals) did not differ (Figure 2). Neither main effects nor their interactions were significant (all $P > 0.05$).

Although neither hormonal priming nor restraint altered the number of crossings (Figure 3), there was a significant effect of the repeated measure of time (e.g. before or after the 5 min restraint or home cage experience; $F_{1,42} = 43.9$, $P \leq 0.05$). Regardless of the type of 5 min experience, the number of crossings declined from the first to the second 10 min monitoring period. There was, however, a significant interaction between type of priming treatment and the type of experience ($F_{2,42} = 3.82$, $P \leq 0.03$). Restrained OO rats had slightly more crossings than their home cage counterparts and restrained EO rats showed fewer crossings than their control. However, none of the posthoc comparisons between restrained and home cage rats were significant (Tukeys, all $P > 0.05$).

Seconds of grooming are shown in Figure 4. There was a significant effect of type of experience ($F_{1,42} = 5.36$, $P \leq 0.03$) and the repeated measure of time ($F_{1,42} = 4.39$, $P \leq 0.05$) but the type of hormone treatment was not significant. Both the time factor and the experience factor reflected an increase in grooming after restraint that was most prominent in OO rats (Tukey's $q_{42,2} = 4.65$, $P \leq 0.05$) and resulted, in part, from a single OO rat that showed excessive grooming after restraint. When this one rat was removed, the mean $\pm$ S.E. for OO restrained rats was 53.65 ± 13.44 , more in line with the grooming evidenced by restrained EP rats. However, even when this rat was removed from the analysis, there was still a significant effect of restraint on the amount of grooming ($F_{1,41} = 4.58$, $P \leq 0.04$).

4.0 Discussion

The current experiment was designed to test the hypothesis that mild restraint would reduce the female's preference to spend time near a sexually active male. Although hormone priming clearly increased male preference in agreement with prior findings (Clark, et al., 2004), there was no effect of the restraint experience on either time with the male or total social time (time with either stimulus animal). These findings contrast with our prior findings that females appeared to avoid contact with a sexually active male by remaining in an experimental "burrow" (Uphouse, et al., 2005) and with the decrease in lordosis behavior seen following restraint (Truitt, et al., 2003; White and Uphouse, 2004). The current findings allow the suggestion that neither sexual motivation nor social motivation were affected by the mild restraint.

Since the lordosis reflex is a consummatory act while partner preference is thought to measure appetitive components of sexual behavior (Avitsur and Yirmiya, 1999; Guarraci and Clark, 2006), different effects of restraint on the two behaviors is not surprising. Although both behaviors rely upon hormonal priming by estrogen and progesterone, the brain areas required for their execution are different. A critical role for the ventromedial nucleus of the hypothalamus in lordosis behavior is well established (Pfaff and Modianos, 1985) while the medial preoptic area and nucleus accumbens may play a more prominent role in female paced mating and/or partner preference (Guarraci and Clark, 2006; Guarraci, et al., 2004; Rivas and Mir, 1990).

Alternatively, the paradigm distinct effects of restraint may have resulted from the absence of physical contact with the male in the partner preference paradigm. Both lordosis assessment and the "burrow" paradigm allowed physical contact with the male (Truitt, et al., 2003; Uphouse, et al., 2005). The act of mating has both aversive and rewarding properties and females exhibit greater male preference under conditions that limit physical contact with the male (Clark, et al., 2004; Guarraci and Clark, 2006). Therefore, we cannot rule out the possibility that restraint accentuated aversive aspects of mating.

However, it is more likely that the different effects of restraint in the “burrow” versus partner preference paradigm resulted from a restraint-induced increase in fear or anxiety that, in the “burrow” paradigm, allowed execution of a behavioral response that was competitive to spending time near the male. In the partner preference paradigm, the female had no “burrow” in which to escape so that competing behavior did not occur. It is, thus, important to note that the number of center crossings did not differ between restrained and nonrestrained females. Therefore, restrained females did not simply escape in a random direction from the center area and remain immobile near one of the stimulus animals. That restraint may have increased fear or anxiety is evidenced by the significant increase in grooming after restraint (Dunn, et al., 1987). Nevertheless, sexual motivation (as measured by time spent with the male) was unaffected. Therefore, while mild restraint reduces the female’s immediate reproductive fitness by reducing the probability of encountering a prospective male (Uphouse, et al., 2005) and reducing the female’s consummatory behavior (White and Uphouse, 2004), sexual motivation, per se, does not appear to be reduced by the mild stress (current study).

Based on these findings, we suggest that restraint induces a state of “fear or anxiety” leading the female to attempt to escape the threat and enter a safer environment. In the forced mating paradigm, the female’s attempt to escape produces fighting and rejection of the male’s advances. The female’s heightened physical activity attempting to escape the male is likely to further exacerbate the HPA axis (Yanagita, et al., 2007). In the “burrow” paradigm, such physical activity does not occur because the female’s escape behavior is successful. With the no-contact partner preference paradigm, fear-motivated behavior would cause the female to leave the open area and move toward the ends of the test apparatus, near one of the incentive animals, and would not interfere with assessment of partner preference. Therefore, mild restraint may reduce the female’s probability of mating but does not appear to reduce female sexual motivation, as measured by her preference for spending time with a male.

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Figure 1. Male preference ratios after hormonal treatment and restraint

Data are the mean $\pm$ S.E. for the ratio between time spent with the male divided by time spent with either the male or female stimulus animal. BEFORE indicates data for all rats during the first 10 min monitoring period. For OO, EO, and EP rats, respectively, data are for 17, 14, and 17 rats. Data for AFTER reflect behavior during the 10 min interval following the 5 min HOME CAGE or RESTRAINT experience. For OO, EO and EP rats in the home cage group, N's are 7, 6 and 7, respectively. For the restraint groups, N's are 10, 8 and 10 for OO, EO, and EP treatments, respectively. * indicates a significant difference relative to OO rats of the same condition.

Figure 2. Total social time after hormonal treatment and restraint

Data are the mean $\pm$ S.E. for the seconds of total social time (time spent with either the male or female stimulus animals). BEFORE indicates data for all rats during the first 10 min monitoring period. Data for AFTER reflect behavior during the 10 min interval following the 5 min HOME CAGE or RESTRAINT experience. N's are as described for Figure 1.

Figure 3. Crossing behavior after hormonal treatment and restraint

Data are the mean $\pm$ S.E. for the number of times females crossed the center of the apparatus. BEFORE indicates data for all rats during the first 10 min monitoring period. Data for AFTER reflect behavior during the 10 min interval following the 5 min HOME CAGE or RESTRAINT experience. N's are as described for Figure 1.

Figure 4. Grooming behavior after hormonal treatment and restraint

Data are the mean $\pm$ S.E. for the seconds that experimental females engaged in self-grooming behavior. BEFORE indicates data for all rats during the first 10 min monitoring period. Data for AFTER reflect behavior during the 10 min interval following the 5 min HOME CAGE or RESTRAINT experience. ** indicates a significant difference relative to same hormone, home cage group. N's are as described for Figure 1.