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PRIOR HORMONAL TREATMENT, BUT NOT SEXUAL EXPERIENCE, REDUCES THE NEGATIVE EFFECTS OF RESTRAINT ON FEMALE SEXUAL BEHAVIOR

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Abstract

These experiments were designed to determine if prior sexual experience reduced the negative effect of mild stress on female sexual behavior. In the first experiment, ovariectomized rats were hormonally primed with estradiol benzoate and progesterone for 3 consecutive weeks during which they received 6 mating experiences in a male's home cage or received no sexual experience. The next week, females were primed with 10 µg estradiol benzoate two days before a 5 min restraint. Both groups were resistant to the negative effects of the stressor. In the second experiment, females received 0, 1, 2, or 3 weeks of 10 µg estradiol benzoate and were restrained on the fourth week after priming with 10 µg estradiol benzoate. Rats without prior hormonal priming showed a decline in lordosis behavior after restraint but prior priming with estradiol benzoate reduced this effect. In the third experiment, rats received 3 weeks of hormonal priming with estradiol benzoate and progesterone with or without sexual experience. An additional group received no sexual experience or hormonal priming. Females were then given a 3-week hormone vacation before testing in the restraint paradigm. All groups showed a decline in lordosis behavior after restraint. The fourth experiment was identical to the third except that sexual experience in the male's cage and in a pacing apparatus were compared. There was no effect of either type of sexual experience on the response to restraint. Possible mechanisms responsible for effects of prior hormonal priming are presented and the absence of an effect of sexual experience is discussed in comparison to findings in male rats.

Keywords

lordosis; ovariectomized rats; proceptivity; stress

1.0 Introduction

In female rats, estradiol and progesterone regulate female reproductive behavior which consists of appetitive, precopulatory and consummatory behaviors [1-3]. Only estradiol is required for lordosis behavior (the consummatory response) while progesterone is required

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for the female to exhibit the entire sexual behavioral repertoire [2, 3]. In particular, progesterone appears to be required for the motivational components of reproductive behavior [4-7]. Such progesterone-facilitated elevation of female sexual motivation may account, in part, for observations that the dose of estradiol required to facilitate lordosis behavior in ovariectomized rats is lower when the hormonal priming includes progesterone [3, 8]. In addition, progesterone has been repeatedly demonstrated to reduce the female's response to stressful stimuli [5, 9, 10] and can attenuate the negative effects of stress on sexual behavior [10, 11]. Thus, progesterone may reduce fear and/or anxiety that are associated with the mating experience [12-14] and thereby enhance hormonal induction of sexual behavior.

Hormonal priming with 10 µg estradiol benzoate restores lordosis behavior in ovariectomized Fischer rats, but without progesterone, females show a reduction in sexual behavior following a brief 5 min restraint experience [10, 11, 15]. Addition of progesterone to the estradiol benzoate priming prevents this inhibition through a mechanism requiring activation of the classical progesterone receptor [11, 15]. Progesterone's ability to reduce the sexual behavioral response to restraint was proposed to result from attenuation of serotonergic changes resulting from the stressor since restraint amplified the ability of a serotonin 1A (5-HT_{1A}) receptor agonist to inhibit lordosis [16]. An inhibitory effect of 5-HT_{1A} receptor agonists on lordosis behavior is well recognized [17, 18] and progesterone attenuates this lordosis-inhibiting effect [19]. Since the act of mating can produce physiological responses similar to those that occur during stress [14], it has been suggested that progesterone's ability to enhance female rat sexual behavior could result from progesterone's anxiolytic/stress-reducing effects [11]. If so, then additional stress-reducing events might also prevent effects of the 5 min restraint on female rat sexual behavior.

Although, in female rodents, sexual behavior appears to be independent of prior sexual experience [20, 21], previous sexual experience has been shown to enhance effects of hormones. For example, the effect of hormones on female rat sexual motivation, measured as the differential latency to traverse a runway to an incentive male or female, was amplified by prior sexual experience [22] and, in female hamsters, the duration of the lordosis response and facilitation of intromission by the male were enhanced in females with prior sexual experience [23]. However, there are few additional studies of the sexual behavioral effects of prior sexual experience in females. Although prior sexual experience has been shown to alter a variety of neural events [24, 25] and to enhance neurochemical responses to a potential mate or to a mating event [26, 27], behavioral effects have seldom been examined. When effects of mating experience on behavior have been examined, most studies have included parturition in the experience and are, therefore, potentially confounded with effects of pregnancy and lactation [28-32].

Progesterone's ability to reduce the sexual behavioral effects of a 5 min restraint experience has been examined only in sexually naive female rats. Given evidence that sexual experience may exert positive effects on reproductive behavior and reduce fear/anxiety, it is possible that prior sexual experience could attenuate the negative effects of a mild stressor on female sexual behavior. The current experiments were designed to test such a possibility.

2.0 Materials and General Methods

All procedures were conducted according to PHS policy and were approved by the IACUC at Texas Woman's University.

2.1 Materials

Estradiol benzoate, progesterone, and sesame seed oil were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Isoflurane (AErrane®) was purchased from Butler Schein Animal Health (Dublin, OH). Decapicone® restrainers were purchased from Braintree Scientific, Inc. (Braintree, MA). Food (Rodent Lab Diet 5001) was obtained from Lab Animal Supply (Highland Village, TX, USA). All other supplies came from Fisher Scientific (Houston, TX).

2.2 General Methods

2.2.1 Animals and Housing—Adult female Fischer rats were purchased from Charles River Laboratories (Wilmington, MA) and housed 2-3 per cage in polycarbonate cages (45.72 × 24.13 × 21.59 cm) with food and water available ad lib. Rats were housed in rooms maintained at 25°C and 60% humidity and with a 12 h-12 h light/dark cycle with lights off at noon.

2.2.2 Surgical procedures and hormonal treatment of animals—After arrival at TWU, females (150-200 g) were anaesthetized with AErrane® and ovariectomized as previously described (White and Uphouse, 2004). Estradiol benzoate was dissolved in sesame seed oil and progesterone was dissolved in propylene glycol. Both hormones were injected subcutaneously (SC) in a volume of 0.1 ml/rat.

2.2.3 Sexual behavioral experience procedures—For experience in the male's cage, females were placed into the home cage (45.72 × 24.13 × 21.59 cm) of a sexually active male for 10 mounts (or a maximum of 10 min) of sexual behavior. For the paced-mating experience, females were pre-adapted to the pacing apparatus (91.44 × 31.75 × 31.115 cm) for 5 min before the introduction of a sexually active male. Thereafter, the female was allowed to interact with the male or escape from the male's chamber for 10 consecutive min.

2.2.4 Restraint and testing procedures—On the day of restraint testing, females were placed into the home cage of a sexually active male and allowed to mate for 10 mounts or 10 min. Thereafter, the female was restrained as previously described [10]. Females were placed head first into a Decapicone® for 5 min and immediately returned to the male's cage for an additional 10 min of testing. Lordosis to mount (L/M) ratios (number of lordosis responses divided by number of male mounts) and lordosis quality scores (relative degree of arching of the back) were scored as previously described [10].

2.2.5 Statistical procedures—L/M ratios and lordosis quality scores were compared by repeated measures ANOVA with type of experience as the independent factor and time relative to restraint as the repeated factor. Data before and after restraint were compared, within experience group, by Dunnett's procedures. Group comparisons after restraint were made with Tukey's test. The statistical reference was Zar [33].

2.4 Specific Procedures

2.4.1 Experiment 1: Effects of sexual experience or hormonal treatment during the 3 weeks preceding restraint—Two weeks after ovariectomy, females were injected with 10 µg of estradiol benzoate. Two days later, rats were injected with 500 µg progesterone in propylene glycol. Rats were divided into two groups for either sexual experience or hormonal treatment only. For rats given sexual experience, four-six hr after the progesterone injection, they were placed into a male's home cage for 10 min of sexual behavioral experience. Forty-eight hr later, rats received a second progesterone injection and a second sexual experience. This sequence was repeated for 3 consecutive weeks. Rats in the

hormonal condition received identical treatment with estradiol benzoate and progesterone but received no sexual experience. On the fourth week of the experiment, all rats were injected with 10 μ g estradiol benzoate; 52-54 hr later, sexual behavior was tested for 10 min in the male's cage. Immediately after this test, females were restrained for 5 min and then returned to the male's cage for 10 consecutive min of behavioral testing.

2.4.2 Experiment 2: Effects of prior treatment with estradiol benzoate on the response to restraint

—Ovariectomized rats were hormonally primed with 10 μ g estradiol benzoate once per week for 0, 1, 2, or 3 consecutive weeks. When estradiol benzoate was not administered, rats were injected with the sesame seed oil vehicle. On the fourth week, rats received 10 μ g estradiol benzoate followed 52-54 hr later with sexual behavioral testing as for Experiment 1 (2.4.1). After this initial test, females were restrained for 5 min and immediately retested for 10 consecutive min.

2.4.3 Experiment 3: Effects of hormonal priming or sexual experience after a 3-week hormone vacation

—Ovariectomized rats were divided into two groups for hormonal treatment or hormonal treatment plus sexual experience as for Experiment 1 (2.4.1). The hormonal group received weekly treatment with 10 μ g estradiol benzoate followed by 2 treatments with progesterone (in propylene glycol) for 3 consecutive weeks. The sexual experience group received sexual experiences two times a week in the male's home cage for 3 consecutive weeks. At the end of the third week, rats were left undisturbed for a 3-week hormone vacation before testing in the restraint paradigm. Ovariectomy of a third group was delayed until week 3 of the experiment so that all females received a 3-week period without hormones. This third group of rats received neither sexual experience nor hormonal treatment. After the 3-week hormone vacation, all rats were injected with 10 μ g estradiol benzoate followed 52-54 hr later with sexual behavioral testing, restraint, and retesting.

2.4.4 Experiment 4: Comparison of two types of sexual experience—Experiment 4 proceeded exactly as for Experiment 3 (2.4.3) with the addition of another sexual experience condition. In this additional condition, rats received sexual experience in a pacing apparatus where the female was allowed to control interaction with the male. All other conditions were as for the prior experiment (2.4.3).

3.0 Results

3.1 Experiment 1: Effects of sexual experience or hormonal treatment during the 3 weeks preceding restraint

The first experiment was designed to determine if prior sexual experience would attenuate the lordosis-inhibiting effects of 5 min restraint. In contrast to the decline in lordosis behavior following restraint in naive rats given a single treatment with 10 μ g estradiol benzoate [10, 11], restraint had no effect on lordosis behavior in rats with 3 consecutive weeks of prior hormonal priming or in rats with hormonal priming plus sexual experience (Figure 1) so that there was no significant difference between the groups (all $p > 0.05$). The means $\pm$ S.E. L/M ratios after restraint in rats given hormonal priming plus sexual experience, respectively, were 1.0 ± 0.01 and 0.96 ± 0.01 . Similarly, lordosis quality did not differ between the groups ($p > 0.05$); for hormonal priming plus sexual experience and hormonal priming only, respectively, means $\pm$ S.E. lordosis quality scores after restraint were 2.69 ± 0.13 and 2.69 ± 0.05 .

3.2 Experiment 2: Effects of prior treatment with estradiol benzoate on the response to restraint

That hormonal priming with or without sexual experience prevented the decline in lordosis behavior after restraint led to the suggestion that the hormonal treatment, and not the sexual experience, was responsible. Therefore, in the next experiment, the effect of estradiol benzoate treatment, alone, was examined. There were three reasons for considering the potential for estradiol benzoate to reduce the effects of restraint.

First, a single treatment with a high dose (50 μ g) of estradiol benzoate is as effective as estradiol benzoate plus progesterone in reducing the sexual behavioral response to restraint [10] and effects of a single estradiol benzoate treatment can endure for one to two weeks [34-36]. Consequently, it was possible that the effects of the 3 weeks of hormonal priming (Figure 1) reflected a cumulative effect of the estradiol benzoate. Second, multiple treatments with estradiol benzoate increase synthesis of progesterone in brain [6] so it is possible that consecutive weeks of hormonal treatment allowed for the presence of progesterone even on the week of restraint when no exogenous progesterone was administered. Finally, prior treatment with estradiol benzoate reduces the lordosis-inhibiting effect of a 5-HT_{1A} receptor agonist, [36-38] and the 5-HT_{1A} receptor has been implicated in the restraint effects [16, 38]

As shown in Figure 1A, prior treatment with even a single 10 μ g estradiol benzoate treatment was sufficient to prevent the restraint from reducing lordosis behavior (Figure 1). Rats given their first estradiol benzoate treatment 52-54 hr before the restraint showed a significant decline in lordosis behavior ($p = 0.05$); groups given estradiol benzoate treatment prior to the week of restraint did not exhibit this decline. Consequently, there was a significant effect of treatment ($F_{3,12} = 19.19$, $p = 0.001$), time relative to restraint ($F_{1,12} = 10.15$, $p = 0.008$) and the interaction between time and treatment ($F_{3,12} = 4.13$, $p = 0.031$). Rats given estradiol benzoate only on the week of restraint were significantly different from all other groups (Tukey's $q_{12,4} = 8.04$, $p = 0.05$).

A similar effect of the prior hormonal treatment was evident when lordosis quality was compared after the response to restraint (Figure 1B). Rats given their first treatment with estradiol benzoate on the week of restraint were the only group showing a decline in lordosis quality (relative to lordosis quality before restraint) ($p = 0.05$) and lordosis quality for this group was significantly lower than the other 3 groups after restraint (Tukey's $q_{12,4} = 7.28$, $p = 0.05$).

Surprisingly, females given multiple weeks of estradiol benzoate treatment showed proceptive behavior (Table 1) that was not reduced by restraint. In contrast, rats given estradiol benzoate only during the week of restraint had a low amount of proceptivity before and after restraint.

3.3 Experiment 3: Effects of hormonal priming or sexual experience after a 3-week hormone vacation

In the next experiment, females received 3 weeks of hormonal priming with estradiol benzoate and progesterone with or without sexual experience in a male's home cage. Females were then left in their home cages and received no additional treatment for 3 consecutive weeks. As a positive control, an additional group of rats (0 weeks) received no previous treatment. On the week of restraint, all rats were injected with 10 μ g estradiol benzoate. Two days later, they were tested for sexual behavior before and after the 5 min restraint experience. As is evident from Figure 2, there was no difference among the three groups of rats and all groups showed a decline in sexual behavior after the restraint. There was a significant effect of time relative to restraint ($F_{1,14} = 37.29$, $p = 0.001$) but no effect of

the type of prior experience ($p > 0.05$). Although lordosis quality declined after restraint ($F_{1,14} = 7.61$, $p = 0.015$), there was no difference among the three groups and no interaction between time and prior treatment ($p > 0.05$) (Table 2). In contrast to rats tested the week after prior hormonal priming, there was no proceptivity in females given a 3-week hormone vacation.

3.4 Experiment 4: Comparison of two types of sexual experience

In the next experiment, the same procedures described above for Experiment 3 (3.3) were repeated but an additional group was added in which females received sexual experience not in a male's home cage but in an apparatus which allowed the female to pace her contact with the male. This experience was added since sexual behavior is reported to be more rewarding when the female is allowed to pace her contact with the male [39, 40]. All females received a 3-week hormone vacation before the week of restraint. As is evident from Figure 3, there was no difference between the sexual experience obtained in the male's cage and in the pacing paradigm (all $p > 0.05$). All groups showed a decline in sexual behavior after restraint ($p = 0.05$). As in prior experiments, restraint reduced lordosis quality in all treatment conditions ($F_{1,28} = 11.712$, $p < 0.002$; Table 3) but the type of prior experience was not a significant factor ($p > 0.05$).

4.0 Discussion

These experiments were designed to determine if prior sexual experience would reduce the lordosis-inhibiting effect of a mild restraint experience. In contrast to expectations, prior sexual experience did not eliminate the effects of restraint; but prior hormonal priming (whether with estradiol benzoate, only, or with estradiol benzoate and progesterone) did significantly reduce the effect of restraint. This effect of hormonal priming was sufficient to account for the absence of a restraint effect when the week of restraint immediately followed prior hormonal priming (Experiment 1, Section 3.1). That hormonal priming, and not sexual experience, was responsible was supported by the last two experiments where females received a 3-week hormone vacation before restraint testing.

In addition to the ability of progesterone to reduce effects of restraint, treatment with a high dose (50 μg) of estradiol benzoate [10], but not 10 μg estradiol benzoate [11], reduces the lordosis-inhibiting effect of restraint. When estradiol benzoate is administered in oil, there is a slow release of the hormone so that its effects can persist for one to two weeks [36, 41]. Thus, the protective effect of multiple injections with 10 μg estradiol benzoate, seen in the current study, may reflect an accumulation of estradiol benzoate and, therefore, a high estradiol benzoate concentration during the week of restraint testing. This possibility is consistent with early studies for amplification of estradiol benzoate priming after prior estradiol benzoate treatments [42, 43].

Alternatively, prior estradiol benzoate treatment may have enhanced endogenous progesterone synthesis before and during the week of restraint. Progesterone is synthesized from pregnanolone by the enzyme 3β -hydroxysteroid dehydrogenase (3β -HSD) [44, 45] and repeated treatment with estradiol benzoate increases the mRNA and protein for 3β -HSD [6]. Thus, estradiol benzoate-induced progesterone synthesis may have been responsible for protection against the restraint. Of relevance to this possibility is the large number of females exhibiting proceptivity (a behavior seldom present in the absence of progesterone) after prior estradiol benzoate treatment (Experiment 2). The absence of such proceptivity when females were allowed a 3-week hormone vacation is consistent with this suggestion.

The lack of an effect of prior sexual experience on either L/M ratios or lordosis quality scores before restraint is consistent with prior observations [26] and with suggestions that

female sexual behavior, unlike male sexual behavior, does not require prior experience [21]. However, the lack of any effect of prior experience on the response to restraint is surprising given the expectation that such experience would reduce the response to stress. It might, though, be important to note that, compared to a plethora of such studies in male rodents [23, 46-48], there have been few such investigations in female rodents where reproductive experience did not include parturition and lactation. Most investigators agree that experience with parturition does have robust anxiety and fear-reducing effects [29, 30, 32]. The current studies provide no evidence that experience with sexual activity, without parturition, altered the female's response to a mild restraint stressor. However, since these studies primarily focused on consummatory components of female sexual behavior, we cannot rule out the possibility that previous sexual experience would alter the female's sexual motivation after restraint. In earlier studies, it was reported that the 5 min restraint experience reduced the time that female spent with the male in a pacing/male contact paradigm [13] but not in the no contact partner preference paradigm [12]. It would, therefore, be interesting to examine the effects of prior sexual experience in these two paradigms.

In summary, 5 min restraint reduced lordosis behavior in ovariectomized females injected with 10 µg estradiol benzoate and this decrease was unaffected by prior sexual experience. These findings contrast with studies in male rodents where prior sexual experience has robust effects on sexual behavior. However, prior hormonal priming did reduce effect of restraint but only when the restraint experience occurred the week after the prior priming. When females were withdrawn from hormones for a period of 3 weeks, the negative effects of restraint were robust and comparable to that of hormone-naïve rats. The mechanisms responsible for the hormonal protection against restraint remain to be delineated but include protective effects of progesterone and are likely to be important to the maintenance of sexual behavior in the naturally cycling female rat. Proestrous females have a relatively low response to stress and exhibit less evidence of anxiety [49, 50]. Proestrous females are also resistant to the lordosis-inhibiting effects of the 5 min restraint experience [16] and current evidence implicates a role for intracellular progesterone receptors in this resistance [11, 15, 51].

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Highlights

The effects of sexual experience on the lordosis-inhibiting effect of restraint were examined.

Three weeks hormonal priming with or without sexual experience reduced the response to restraint.

A three-week hormone vacation eliminated the effects of the prior experience.

There was no effect of sexual experience.

Figure 1. Effects of prior estradiol benzoate priming on the response to restraint

Ovariectomized rats were injected with 10 μ g estradiol benzoate for 0, 1, 2, or 3 weeks. On the fourth week, all rats were injected with 10 μ g estradiol benzoate and tested two days later before and after a 5 min restraint experience. Data in Figure 1A are the mean $\pm$ S.E. for L/M ratios before and after the restraint experience. Ns for 0, 1, 2, and 3-week groups are 5, 4, 4, and 3. Data in 1B are the mean $\pm$ S.E. for lordosis quality scores before and after the restraint experience. Ns for 0, 1, 2, and 3 week groups are 5, 4, 4, and 3. * indicates a significant difference after restraint relative to that before restraint and also a significant difference from the other groups, after restraint.

Figure 2. A 3-week hormone vacation eliminates effects of prior hormonal priming and sexual experience on the response to restraint

Ovariectomized rats were given 3 consecutive weeks of hormonal priming with 10 μ g estradiol benzoate and 500 μ g progesterone or 3 weeks of hormonal priming plus sexual experience. Sexual experience took place 2 times per week in the home cage of a sexually active male. A third group received neither hormonal priming nor sexual experience. After the prior experience, females received a 3-week hormone vacation before the week of restraint testing, when females were injected with 10 μ g estradiol benzoate and restrained two days later. Data are the mean $\pm$ S.E. L/M ratios before and after the restraint experience. Ns for 0 week, hormone group and experience in male's cage, respectively, are 6, 6, and 5. * indicates a significant effect of restraint, within treatment, relative to that before restraint.

Figure 3. Comparisons of sexual experience in the male's cage with that in a pacing apparatus Ovariectomized rats were given 3 consecutive weeks of hormonal priming with 10 μ g estradiol benzoate and 500 μ g progesterone or 3 weeks of hormonal priming plus sexual experience. Sexual experience took place 2 times per week in the home cage of a sexually active male or in a pacing apparatus where females controlled the frequency of contact with the male. A fourth group received neither hormonal priming nor sexual experience. After the prior experience, females received a 3-week hormone vacation before the week of restraint testing, when females were injected with 10 μ g estradiol benzoate and restrained two days later. Data are the mean $\pm$ S.E. L/M ratios before and after the restraint experience. Ns for 0 week, hormone group, experience in the male's cage, or experience in the pacing apparatus, respectively, are 11, 11, and 5, and 5. * indicates a significant effect of restraint, within treatment, relative to that before restraint.

Table 1

Proceptivity and resistance after restraint in rats given prior estradiol benzoate treatment

	0 Weeks	1 Week	2 Weeks	3 Weeks
N	3	5	4	4
% Proceptive Before Restraint	33.3	60	75	100
% Proceptive After Restraint	33.3	40	100	100

Table 2

Lordosis quality after a 3-week hormone vacation

	0 Weeks	Hormonal Priming	Sexual Experience
N	6	6	5
Lordosis Quality Before Restraint	2.88 ± 0.043	2.67 ± 0.153	2.63 ± 0.172
Lordosis Quality After Restraint	2.53 ± 0.149	2.35 ± 0.31	2.19 ± 0.19

Table 3

Lordosis quality after two types of sexual experience

	0 Weeks	Hormonal Priming	Sexual Experience in Male's Cage	Sexual Experience in Pacing Apparatus
	11	11	5	5
Lordosis Quality Before Restraint	2.66 ± 0.125	2.72 ± 0.08	2.86 ± 0.134	2.61 ± 0.23
Lordosis Quality After Restraint	2.07 ± 0.16	2.28 ± 0.187	2.67 ± 0.22	2.20 ± 0.313