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Research report

Prior hormonal treatment, but not sexual experience, reduces the negative effects of restraint on female sexual behavior

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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 3-week hormone vacation eliminated the effects of the prior experience.
- There was no effect of sexual experience.

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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 six 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.

1. 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 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

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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. 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 12–12 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 [10]. 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].

2.3. Specific procedures

2.3.1. 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, 4–6 h after the progesterone injection, they were placed into a male's home cage for 10 min of sexual behavioral experience. Forty-eight hour 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 μg estradiol benzoate; 52–54 h 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.

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

Ovariectomized rats were hormonally primed with 10 μg 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 μg estradiol benzoate followed 52–54 h later with sexual behavioral testing as for Experiment 1 (Section 2.3.1). After this initial test,