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Vaginocervical stimulation attenuates the sensitization of appetitive sexual behaviors by estradiol benzoate in the ovariectomized rat

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ABSTRACT

The acute administration of estradiol benzoate (EB) to the ovariectomized (OVX) rat induces low levels of lordosis while sexually appetitive behaviors (e.g., hops, darts, solicitations) are absent, yet the repeated administration of EB results in a behavioral sensitization in which lordosis is potentiated and sexually appetitive behaviors are induced. We have shown that repeated copulation attenuates the sensitization of appetitive sexual behaviors. Here, we assessed which component of male stimulation during copulation is involved in the attenuation. On 8 occasions, sexually experienced OVX Long–Evans rats were treated with 10 μg EB and 48 h later assigned to one of six groups that differed in their experience on intermediates tests (2–7). One was given repeated access to a male (EB/Male), and another was placed in the copulation chamber alone (EB/Alone) on intermediate tests. Three groups were given one of three somatosensory stimuli by the experimenter: manual flank stimulation (FLS), clitoral stimulation (CLS), or vaginocervical stimulation (VCS). Finally, the control group was left undisturbed in the animal care facility (ACF). Sexual behaviors were measured on Tests 1 and 8. VCS received from the experimenter (VCS) or from the male during copulation (EB/Male) attenuated the magnitude of the sensitization of appetitive sexual behaviors compared with those that were not brought to the testing rooms (ACF), and the effect was most pronounced on sexual solicitations. These results suggest that VCS received during penile intromission inhibits the sensitization of sexually appetitive behaviors by repeated administration of EB. As such, repeated administration of EB may oppose those mechanisms that induce estrous termination, perhaps by sensitizing inhibitory processes within the ventromedial hypothalamus that typically prevent the display of sexual behaviors (i.e., by facilitating disinhibition).

Introduction

Sensitization of sexual behaviors occurs in the ovariectomized (OVX) rat following the repeated administration of estradiol alone. The acute administration of 5 or 10 μg estradiol benzoate (EB), without subsequent progesterone priming, induces low levels of lordosis and very few, if any, sexually appetitive behaviors (e.g., hops, darts, ear wiggles, solicitations). However, its repeated administration induces a behavioral sensitization, potentiating lordosis and activating sexually appetitive behaviors. We have previously shown that the administration of 10 μg EB 48 h prior to copulation every four days results in a robust behavioral sensitization (Jones et al., 2013; Jones and Pfaus, 2014), but that the effect on appetitive behaviors is greater when EB is repeatedly administered in the absence of copulation (Jones and Pfaus, 2014). Since repeated copulation attenuates the sensitization of appetitive behaviors, it is possible that somatosensory stimulation received from the male during mating inhibits this sensitization.

During a typical copulatory bout, which begins with anogenital sniffing from either animal, the female will typically entice the male to chase and mount her by displaying partial (i.e., hopping and darting) or full solicitations (defined as a headwise orientation toward the male followed by a runaway, referred to here as solicitations), which occur in proximity or more distal to the male, respectively (McClintock, 1984; Pfaus, 1996). Solicitational behaviors entice the male to mount (Erskine, 1989) which result in palpation of the flanks, and when coupled with thrusting, stimulates the anogenital region including the clitoris (Pfaff et al., 1977; Pfaus et al., 2014). Solicitations also increase the probability of intromission (Erskine, 1989; McClintock and Adler, 1978), which occurs when the erect penis penetrates the vagina during a mount with a single deep thrust (Bermant, 1965), stimulating the external and interval vagina and presumably the cervix. Ejaculation occurs following approximately 7–9 intromissions and provides strong and sustained stimulation of the vagina and cervix by the deposit of the copulatory plug which congeals and protects sperm transport (Toner et al., 1987). Behaviorally the female holds the lordosis posture while the male's pelvis is maintained in close contact with the perineal region (Bermant, 1965). The female

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will then typically engage in a bursting dismount and run away from the male (Pfaus, 1996; Pfaus et al., 2014). A refractory period follows, lasting a few minutes, before the female revisits the male and solicits him to stimulate mounting and a new bout begins. Thus, during copulation the female receives flank, clitoral, and vaginocervical stimulations from the male.

Sensory stimulation of the genitals that is received during mating can potentiate or inhibit sexual behavior. The pattern of clitoral stimulation (CLS) for example, can facilitate or reduce sexually appetitive behaviors, depending on the pattern of stimulation, such that solicitations are facilitated when an experimenter applies continuous stimulation prior to copulation, and reduced when applied in a distributed manner (Cibrian-Llanderal et al., 2010). Similarly, vaginocervical stimulation (VCS), induced by penile intromissions, or experimentally applied with a glass rod, potentiates sexual behavior at the beginning of a mating session (Bennett et al., 2001; Foreman and Moss, 1977; Rajendren et al., 1991; Rajendren and Moss, 1993). However, as the amount of VCS increases, sexually appetitive behaviors decline coincident with an increase in defensive behaviors in response to mounts, prior to a decline in lordosis frequency and magnitude, as the female gradually enters estrous termination (Erskine and Baum, 1982; Erskine et al., 1989; Hardy and Debold, 1972; Lodder and Zeilmaker, 1976; Pfaus et al., 2000). The receipt of 10–15 VCS accelerates the onset of estrous termination (Coopersmith et al., 1996; Hardy and Debold, 1971; Lodder and Zeilmaker, 1976; Pfaus et al., 2000) and pseudopregnancy (Adler, 1969; Erskine et al., 1989; Frye and Erskine, 1990; Lehmann and Erskine, 2004). However, frequent hormone priming offsets the ability of VCS to induce estrous termination (Pfaus et al., 2000) and reduces the activation of glutamatergic signaling in the vlVMH which is associated with inhibition of sexual behavior (Georgescu et al., 2009, 2012, 2014; Georgescu and Pfaus, 2006a,b), suggesting that the mechanisms of repeated hormone administration act in opposition to those that are inhibitory to sexual behavior. Given that VCS is a well-known inhibitory stimulus to sexual behavior, and that frequent hormone priming offsets that inhibition, we selected a stimulation frequency that is known to induce estrous termination and pseudopregnancy following VCS. The stimulation pattern was kept constant in all manual stimulation groups.

Since varying forms of somatosensory stimulation during mating have been shown to both facilitate and inhibit sexual behavior, the aim of the current study was to mimic the stimulation received from the male by experimentally-applying 15 VCSs, CLSs, or flank stimulations (FLSs), to examine whether any of those somatosensory components contribute to the attenuation of the sensitization of sexual behavior by repeated administration of EB, as occurs with repeated copulation (Jones and Pfaus, 2014). Since we also previously reported that repeatedly treating the OVX rat with EB and placing her in the testing chamber alone was more effective at inducing sensitization compared with those that copulated (Jones and Pfaus, 2014), we also assessed whether sensitization would occur if females were treated with EB but left in their home cages in the animal care facility (ACF). It was hypothesized that 1) all groups would display behavioral sensitization to EB, and that 2) a greater increase in sexually appetitive behaviors would be observed in the two groups that did not receive somatosensory stimulation (i.e., ACF and EB/Alone groups) compared with each of the stimulation groups, particularly those that received VCS from the male (EB/Male) or experimenter (VCS).

Materials and methods

Animals

Two cohorts of 36 Long–Evans females (200–250 g) were purchased from Charles River, St-Constant, QC, Canada three months apart, and housed in pairs in clear Plexiglas shoebox cages lined with either beta chip or corncob bedding. A group of 36 Long–Evans males (300–350 g) were also purchased from the same supplier, housed in groups of four in clear Plexiglas cages lined with beta chip, and used as stimulus males for both cohorts. Prior to the experiment, males were given 4 sexual experience sessions in the 4-hole unilevel pacing chamber with stimulus OVX females primed with estradiol benzoate and progesterone. Each cohort included 6 females per group, for a final sample size of 12 females per group (N = 72).

Ovariectomy

All animals were given one week to acclimate to the ACF. Females were then bilaterally ovariectomized (OVX) via a single lumbar incision under a mixture of 4:3 ketamine hydrochloride (50 mg/mL; Ketaset©, Wyeth Canada) and xylazine (4 mg/mL; Rompum©, Bayer Healthcare, Canada), injected IP (1 mL/kg). They were then identified by ear punch and given SC injections of PenG (0.1 mL/rat) and 5 mg/kg/mL Enrofloxacin (Baytril©) and rehydrated with 2 mL of saline. They were given one-week post-operative recovery, as in Jones et al. (2013) prior to sexual training.

Hormone preparation

Estradiol benzoate (EB; 10 μg) and progesterone (P; 500 μg) were dissolved in 0.1 mL reagent grade sesame oil administered SC, 48 h and 4 h prior to testing respectively. Steroid hormones were received from Steraloids (Hanover, NH).

Apparatus

All behavioral training and tests occurred in unilevel 4-hole pacing chambers, which are bisected with a Plexiglas divider with four square holes cut in the bottom that are large enough for the female to cross through but too small for the male (Jones et al., 2013). The dustpans of the chambers were lined with beta chip and rested below a metal grid floor elevated approximately 2.54 cm. Both the grid and the 4-hole partition were removed for groups receiving manual stimulation to prevent females escaping the experimenter's grasp. All tests took place in chambers where animals had previously copulated.

Training and testing procedures

All training and tests occurred during the middle third of the dark cycle. Females were injected with EB followed by P prior to each of four sexual training sessions occurring at 4-day intervals and given a 2-week hormone washout (as in Jones et al., 2013; Jones and Pfaus, 2014). Females were then treated with EB/Alone, at 4-day intervals for the remainder of the experiment. For each copulatory session, the male was placed on the right hand side of the chamber, and allowed a 5-minute acclimation period prior to introducing the female. The animals were then left undisturbed for 30 min. All copulatory tests were recorded using a Sony Handycam digital camera and scored using the Behavioral Observation Program (Cabilio, 1996).

All animals copulated on Tests 1 and 8, and groups of animals received one of six experimental manipulations on Tests 2 through 7: one group copulated with a sexual experienced Long–Evans male in a 4-hole pacing chamber on all tests (EB + Male), a second was placed in the 4-hole pacing chamber alone (EB/Alone), and a third group was left in their home cage in the ACF. The three remaining groups received experimenter-applied vaginocervical (VCS), clitoral (CLS), or flank (FLS) stimulations as described in detail below. For these groups, the female was first placed in a testing chamber alone, and a 30-minute timer was set. After 1 min had lapsed, the first stimulation (described below) was given, and each subsequent stimulation was applied every 2 min thereafter, for a total of 15 stimulations over 30 min. Following the final stimulation, animals were left in the chamber until the 30 min had elapsed (i.e., <1 min) before returning to their home cage.