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Research report

Repeated administration of estradiol promotes mechanisms of sexual excitation and inhibition: Glutamate signaling in the ventromedial hypothalamus attenuates excitation

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HIGHLIGHTS

- Repeated estradiol treatments sensitize sexual behaviors in ovariectomized rats.
- Copulation and vaginocervical stimulation attenuate the sensitization.
- We show that AMPA receptor activation in the vlVMH also attenuates sensitization.
- Estrous termination is accelerated by repeated estradiol in the absence of copulation.
- Estradiol promotes the activation and inhibition of female sexual behavior.

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ABSTRACT

Repeated administration of 10 μg of estradiol benzoate (EB) every 4 days to the ovariectomized (OVX) rat induces a behavioral sensitization of sexual behaviors. Repeated copulation or the receipt of vaginocervical stimulation (VCS) attenuates the sensitization of appetitive sexual behaviors, suggesting that VCS acts in opposition to the mechanisms that induce the sensitization. It is known that VCS accelerates the onset of estrous termination (characterized by a decrease in appetitive sexual behaviors, and an increase in defensive behaviors prior to the decline in lordosis), and glutamate transmission in the ventromedial hypothalamus (VMH), particularly via AMPA receptor signaling, is an important regulator of this effect. Thus, the current studies examined whether mechanisms of estrous termination are involved in the attenuated sensitization to EB that occurs with repeated copulation. In the first study, OVX rats received infusions of AMPA to the VMH on tests 2–4, and sexual behavior was measured on tests 1 and 5. Appetitive sexual behaviors were lower in females that received AMPA infusions in place of copulation compared to saline, suggesting that AMPA receptor activation by VCS may be playing a role in the attenuation of sensitization. In the second study, females that were not given the opportunity to copulate on tests 2–4 fell out of behavioral estrus faster than those that did, suggesting that both excitatory and inhibitory mechanisms of sexual behavior become sensitized with repeated administration of EB. Together these findings extend our hypothesis that repeated episodes of heat sensitize the activation of sexual behaviors to increase the probability of eventual fertilization.

1. Introduction

The acute administration of estradiol benzoate (EB) to the ovariectomized (OVX) rat induces a low frequency of lordosis in response to mounts from a male, and very few sexually appetitive behaviors such as hops, darts, solicitations, and ear wiggles. Sexually appetitive behaviors are reliably and maximally induced, and lordosis is potentiated, 4 h following an acute injection of progesterone (P) administered 36–48 h following the EB injection [5]. However, chronic administration of 10 μg EB alone every

Abbreviations: EB, estradiol benzoate; LM, lordosis magnitude; LQ, lordosis quotient; MeA, medial amygdala; mPOA, medial preoptic area; OVX, ovariectomy; P, progesterone; PAG, periaqueductal gray; pdMeA, posterodorsal division of the medial amygdala; PBS, phosphate buffered saline; VCS, vaginocervical stimulation; vlVMH, ventrolateral division of the ventromedial hypothalamus; VMH, ventromedial hypothalamus.

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four days results in a behavioral sensitization such that following the fourth injection, lordosis is maximally induced and sexually appetitive behaviors occur more frequently compared to the initial injection and reach a plateau [22]. This behavioral sensitization occurs independently of adrenal P [22]. Interestingly, sensitization of appetitive behaviors is attenuated if females copulate [23], or receive vaginocervical stimulation (VCS) during each episode of heat (either from penile intromission or experimenter-applied; [24]). VCS is also inhibitory to the duration of sexual behavior, by accelerating the onset of estrous termination, which is characterized by a decrease in sexually appetitive behaviors with an increase in defensive behaviors prior to the decline in lordosis frequency and magnitude [41]. Thus, here we investigated whether mechanisms associated with estrous termination are also involved in the attenuated sensitization of appetitive sexual behaviors that occurs with repeated copulation.

Glutamatergic signaling in the ventrolateral portion of the ventromedial hypothalamus (vlVMH) inhibits sexual behavior yet the inhibition is delayed or reduced if females are primed with EB. Presenting the OVX rat with a sexually vigorous male induces glutamate release into the vlVMH, and the release is attenuated by prior treatment with EB [15]. Moreover, sexual behavior is facilitated in OVX EB-primed rats by infusions of GABA-a agonists [31,33], or glutamate receptor antagonists [18] into the vlVMH, whereas infusions of glutamate or its ionotropic receptor agonists rapidly inhibit lordosis [19,25,32] and hops and darts, while activating defensive behaviors [19], similar to the behavioral pattern characteristic of estrous termination. That inhibitory effect was most robustly induced by AMPA [19]. Importantly, that endogenous glutamate within the VMH is inhibitory to lordosis and under the control of GABA and steroid hormones, has also been supported electrophysiologically in tissue slices examined across the natural estrous cycle [6]. The onset of estrous termination is accelerated by the receipt of VCS from penile intromissions or experimenter applied with a glass rod (e.g., [12,30,41]), however if the frequency of hormone priming is increased from every 28 days to every 14 days to every 4 days, the inhibitory effect of VCS becomes progressively less robust [41]. The administration of VCS also activates Fos, a marker of neuronal activation, within numerous sexually relevant brain regions [40], including glutamate neurons of the vlVMH [20], but the number of Fos-labeled glutamate cells in the vlVMH following VCS is reduced by EB + P priming compared to oil treated controls [20]. Importantly, the ability of VCS to induce estrous termination is prevented by blocking the AMPA/kainate glutamate receptor subtype [16]. Together those data suggest that EB prevents the inhibition of sexual behavior by VCS that appears to be mediated, at least in part, by glutamate transmission and more specifically the activation of AMPA/kainate receptors within the vlVMH.

Because VCS attenuates the sensitization of sexually appetitive behaviors by EB, the first behaviors that decline in frequency as estrous termination sets in [42], the overarching goal of these studies was to examine whether mechanisms related to estrous termination are involved in the attenuation of appetitive behaviors in females that are repeatedly treated with EB and given the opportunity to copulate on every test. Since glutamate and its ionotropic receptor agonists infused in the vlVMH inhibit sexual behavior in a pattern reminiscent of estrous termination [19], and antagonizing the AMPA receptor subtype blocks the effect of VCS on estrous termination [16], in the first experiment we hypothesized that AMPA infusions to the vlVMH would mimic the effect of repeated copulation in females repeatedly treated with EB, resulting in an attenuation of EB sensitization compared to vehicle infused animals. In the second study we hypothesized that estrous termination would be accelerated in females that copulate during each episode of heat (i.e., that repeatedly receive VCS through copulation) compared to those restricted from copulating on intermediate tests.

2. Materials and methods

2.1. Animals

Animals were purchased from Charles River Canada (St-Constant, Québec, Canada), and given one week to acclimate to our facilities. Females were housed in pairs in clear Plexiglas® cages lined with a mixture of corncob and sanichip bedding (females), or in groups of four in large gang cages lined with sanichip (males). Food (Charles River, 5075) and tap water were freely available. Rooms were maintained at 21 °C, on a 12-h reverse cycle (lights off at 8 AM). Following approximately one-week acclimatization to the animal facility, females were OVX and males were given four sexual training sessions in the pacing chambers with a separate group of OVX stimulus females primed with EB + P. Two cohorts of animals were used for the AMPA study occurring at 3-month intervals. A group of sexually experienced OVX rats, used in unrelated studies, were used as stimulus females in the estrous termination study.

Animal pain and discomfort was minimized throughout the duration of the animal's stay in our facilities. All experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care, and approved by the Concordia University Animal Research Ethics Committee.

2.2. Surgeries

2.2.1. Ovariectomy

Ovaries were removed bilaterally following a 1 mL/kg IP injection of a 4:3 mixture of ketamine hydrochloride (50 mg/mL; Ketaset ©, Wyeth Canada) and xylazine hydrochloride (4 mg/mL; Rompum©, Bayer Healthcare). Animals were also ear punched for identification purposes, and post-operative care was given with SC injections of PenG (0.1 mL/rat) and 2.5 mg/kg/mL of flunixin meglumine (Banamine©), and rehydrated with 2 mL of saline administered SC. Animals were given one week post-operative recovery prior to sexual behavior training.

2.2.2. Cannulation

Cannulations occurred within 3 days of the final sexual training session (described below), during the 2-week hormone washout period. Females were anesthetized using an isofluorane:oxygen gas mixture (2.5–5% isofluorane:0.8 L/min O_2) and placed into a Kopf stereotaxic instrument. A mixture of 0.05% lidocaine (CDMV-3913) and 0.05% marcaine (0.25%, CDMV-95865) in an 8.4% sodium bicarbonate solution (CDMV-93-500-EV) was injected below the scalp prior to incision. Painkiller (0.2 mL Anafen) and antibiotic (0.2 mL PenG) were then injected SC, and 2 mL of saline was administered both before and after surgery for rehydration purposes, and eyes were kept hydrated with an ocular gel (Natural Tears®, Alcon). Using the flat skull technique, bilateral guide cannula (C232G-1.5/SPC 11 mm; Plastics One) were then implanted targeting the vlVMH according to the following coordinates: AP 2.6 mm, ML ±0.75 mm, DV 8.4 mm. Guide cannulae were fixed to the skull by three stainless steel screws and dental cement. The dummy cannula (C232DC-1.5; Plastics One, Roanoke, VA) was then inserted, extending 0.5 mm beyond the guide, and covered with a dust cap (303DC/1; Plastics One, Roanoke, VA). Finally, Polysporin® was applied to the incision site. Infusion cannulae (C232i-1.5/SPC; Plastics One, Roanoke, VA) extended 1 mm beyond the guide.