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The inhibitory effects of corncob bedding on sexual behavior in the ovariectomized Long–Evans rat treated with estradiol benzoate are overcome by male cues

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ABSTRACT

The mechanisms underlying the sensitization of sexual behaviors by repeated administration of estradiol benzoate (EB) to ovariectomized (OVX) rats are not well understood. Here we tested whether two housing conditions play a role. Sexual behavior in the female rat is dependent on the activation of ERα (estrogen receptor alpha) by estradiol. Corncob (CC) bedding has been reported to have adverse effects on the reproductive behavior and physiology of rats, and to disrupt ERα signaling in mice. In addition, some rodent behaviors are stimulated by olfactory stimuli and enhanced in the presence of estradiol. Upon arrival to the facilities OVX Long–Evans rats were housed on either Sani-Chips (SC) or CC in a room that housed only females (F) or males and females (M). Females were first given four sexual training sessions with 10 μg EB + 500 μg progesterone (P; administered 48 h and 4 h prior to training, respectively), followed by a 2-week hormone washout period. Next, 10 μg EB was administered s.c. every 4 days, 48 h prior to each of 8 test sessions in a unilevel 4-hole pacing chamber. On the final training day (i.e., when primed with EB + P), no inhibitory effects of corncob bedding were found, however a facilitation of the lordosis quality occurred in SC/F. Although all groups appear to have sensitized to the repeated administration of EB, CC/F animals displayed fewer high quality lordosis magnitudes and hop/darts, and received fewer mounts and intromissions overall. They also had a lower lordosis quotient (LQ) on tests 2–4 although this effect disappeared by test 5. These results suggest that although CC may inhibit some components of female sexual behavior when primed with EB alone, cues from sexually vigorous males can overcome that inhibition. Moreover, they suggest that male cues can facilitate mechanisms of estradiol sensitization. We recommend that quality control studies be conducted at individual institutions to assess any impact of corncob bedding on animal physiology and behavior.

Introduction

Ovariectomized (OVX) rats are behaviorally more sensitive to subsequent injections of estradiol benzoate (EB) administered alone compared to the initial injection (Babcock et al., 1988; Beach and Orndoff, 1974; Blaustein et al., 1987; Clark and Roy, 1983; Gerall and Dunlap, 1973; Jones et al., 2013; Kow and Pfaff, 1975; Parsons et al., 1979; Whalen and Nakayama, 1965). Whereas the acute administration of EB alone partially activates lordosis, repeated administration potentiates lordosis and activates sexually appetitive behaviors (such as solicitations, hops/darts and ear wiggles). Recently we examined the time-course of this behavioral sensitization using three doses of EB that are commonly used to induce sexual behavior in the OVX rat. We found that in two strains of rats (Long–Evans and Wistars), sensitization occurs following administration of 5 or 10 μg EB every four days (Jones et al., 2013). Sensitization is most robust if females are treated with 10 μg EB but not given the opportunity to copulate on every test (Jones and Pfaus, 2014). Moreover, in the absence of the opportunity to copulate, sensitization also occurs using 2 μg EB (Jones and Pfaus, 2014), a dose that had previously been reported to be subthreshold at inducing sexual behavior in Long–Evans without subsequent progesterone treatment. We are currently investigating the mechanisms that underlie this effect, using the dose that induced the most robust sensitization (10 μg). In the present study we examined whether two housing factors might interfere with the effect.

Recently our animal care facility considered implementing corncob bedding in home cages. The primary advantage of this bedding is greater absorbency, which reduces bacterial growth and ammonia levels (Burn and Mason, 2005). The bedding is also beneficial to staff, since dust and allergens are reduced, and due to its increased absorbency, requires less frequent cage changes compared to wood chip bedding. However some groups have reported adverse effects of housing animals on corncob. Within two weeks, male and female (ovary-intact and OVX)

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rat sexual behavior was inhibited and ovarian cyclicity was disrupted (Mani et al., 2005; Markaverich et al., 2002a,b, 2005, 2007a), effects that were reversed when returned to woodchip bedding or wire-mesh cages (Markaverich et al., 2002a). Although corncob is plant-based and may act as a phytoestrogen, the compound THF-diol (tetrahydrofuran diols), which appears to be the inhibitory agent in corncob bedding, does not compete for estrogen receptor (ER) binding sites in uterine tissue (Markaverich et al., 2002a,b). However, Villalon Landeros et al. (2012) recently reported that male Californian mice housed on corncob exhibited differences in estrogen receptor alpha (ERα) signaling pathways in a number of brain regions associated with aggression, many of which are also implicated in rat sexual behavior. For example, they found fewer ERα-positive cells in the ventromedial hypothalamus (a region critical in the expression of lordosis, the concave arching of the back that permits penile intromission) and reduced numbers of phosphorylated ERK in the medial preoptic area and medial amygdala (regions that are implicated in sexually appetitive behaviors). Together these data suggest that compounds within corncob bedding do not act through ERs, but merge and disrupt downstream ER signaling pathways. This is problematic for studies investigating endocrine control of behavior. Given the implications this could have on the underlying mechanisms associated with the sensitization of sexual behaviors by chronic EB, we tested whether bedding type would alter this sensitization.

Olfactory cues from males might also affect the sensitization of female sexual behavior by repeated EB. Many rodent behaviors can be strongly influenced by olfactory cues, and enhanced in the presence of estradiol. For example the exposure of nulliparous and/or OVX female rats to pup odors can stimulate maternal behavior (Mayer and Rosenblatt, 1980), and the facilitation of maternal behaviors in OVX pregnancy-terminated (gestational day 15) dams is accelerated in the presence of estradiol (Stolzenberg et al., 2009). Olfactory stimuli from a sexually vigorous male may also be involved in mating-induced enhancement of sexual behaviors in estradiol-primed OVX rats repeatedly mated within an episode of heat (Rajendren et al., 1990). Moreover, female rats in estrus have an unconditioned preference for a sexually vigorous male (Agmo, 1999; Sakuma, 2008), an effect dependent on the presence of estradiol, and an intact medial preoptic nucleus (Sakuma, 2008; Xiao et al., 2005). Thus, if reducing exposure to male cues (e.g., olfactory, auditory, visual) (by housing the sexes in separate rooms), influences the development of the behavioral sensitization to EB, it may help pinpoint the underlying neural pathways involved.

The current study examined whether housing females on corncob versus Sani-Chips bedding, or whether housing males and females in separate colony rooms, would alter sexual behavior in fully primed (10 μg EB + 500 μg P) females, or the sensitization of sexual behaviors following repeated EB injections alone (10 μg).

Materials and methods

This experiment was conducted in accordance with the ethical standards established by the Canadian Council on Animal Care (CCAC), and approved by Concordia University's Animal Care Committee.

Animals

Long–Evans female rats weighing approximately 150–200 g were purchased from Charles River Laboratories (St-Constant, Quebec) where they were housed on Beta Chip® until weaning, then group housed in stainless steel cages with mesh bottoms. Upon arrival to our facilities, they were housed in pairs in clear Plexiglass chambers lined with either Sani-Chips (category 7090A, Harlan, Montreal, Quebec) or corncob bedding (1/4″ category 7097, Harlan, Montreal, Quebec) until the end of the experiment (total of 11 weeks). All colony rooms were maintained on a 12-hour reversed light cycle (lights on at 8 PM) at approximately 21 °C. Animals had ad lib access to standard laboratory chow (Charles River #5075) and tap water. They were given a week of acclimatization to the animal facilities and handled daily from days 4 to 7, when they were OVX.

Sexually experienced Long–Evans males (housed 3–4/cage) obtained from the same supplier, were housed on Sani-Chips bedding, and used as stimulus animals for training and test sessions. Each male was only used once on each training or test day, and assigned randomly to the female. Whereas females were strictly returned to their assigned colony room, males were returned at random (but never to a "female-only" room).

Ovariectomy

Ovariectomies (OVX) were performed following an i.p. injection (1 mL/kg) of ketamine (50 mg/mL; 50 mg/mL; Ketaset©, Wyeth Canada) and xylazine hydrochloride (4 mg/mL; Rompum©, Bayer Healthcare) mixed at ratio of 4:3 respectively. When animals were no longer responsive to a foot pinch, the eyes were lubricated with drops (Natural Tears, Alcon), and ears were punched for identification purposes. They were then bilaterally OVX via a single lumbar incision. Polysporin was applied to the incision site, and Flunixin Meglumine 2.5 mg/kg/mL (Banamine©) and 5 mg/kg/mL Enrofloxacin (Baytril©) were given by s.c. injection. Animals were next hydrated with 1 mL 0.9% saline injected s.c., and given one week to recover.

Hormones

Estradiol benzoate (10 μg) was dissolved in 0.1 mL of sesame oil, and injected s.c. 48 h prior to each training and test session. Progesterone (500 μg) was dissolved in 0.1 mL sesame oil and injected s.c. 4 h prior to each training session.

Treatment conditions

One of the more practical advantages of using corncob bedding is it requires less frequent cage changes compared to woodchip beddings. One potential explanation for disrupted sexual behavior reported in animals housed on corncob bedding is the frequency of cage changes, and subsequently less frequent handling (Castelhano-Carlos and Baumans, 2009). For example, locomotor activity and grooming behaviors are increased on cage cleaning days (Saibaba et al., 1996), and increased heart rate, blood pressure (Duke et al., 2001), and corticosterone (De Boer et al., 1990) are observed following handling procedures. Good laboratory practices include handling of animals to minimize stress, by reducing the novelty of the experimental conditions. Novelty and stress disrupt sexual behavior in rodents (Pfaus and Wilkins, 1995; Yoon et al., 2004), and cage cleaning induces a stress response (Castelhano-Carlos and Baumans, 2009). Since corncob bedding requires less frequent cage changes, variations in handling and stress responses may account for these differences. Therefore, to control for the potential confound, cage change frequency was experimentally controlled. Sani-Chips bedding was changed on a bi-weekly schedule, whereas corncob bedding was changed on a weekly schedule. However females housed on corncob also received a "sham-cage change", such that they were simply lifted out of their cage, and placed back in, on days coinciding with the second cage change of Sani-Chips animals. This ensured that handling was consistent between all groups. All cage changes were done in the afternoon (i.e., never prior to training or testing) to minimize the potential impact of cage changes on test days.

To determine whether the presence of males housed in the same room as females would affect the sensitization of female sexual behavior, females on each bedding type were housed in either a female-only room or a room housing both males and females. Hence, this experiment consisted of a total of four groups according