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Behavioral defeminization by prenatal androgen treatment in rats can be overcome by sexual experience in adulthood

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ABSTRACT

Exposure to testosterone during a critical period of prenatal development disrupts the normal display of sexual behaviors in adult ovariectomized (OVX) rats treated with estradiol benzoate (EB) followed by progesterone (P). The organizational hypothesis posits that prenatally androgenized females (PNAFs) are desensitized to EB. We tested this hypothesis by first treating PNAFs with varying doses of EB (2.5, 5, 10, 20 μg) followed by P (500 μg), and second by subjecting females to an established EB behavioral sensitization paradigm where females are first given sexual experience with EB (10 μg) and P prior to repeated sexual behavior testing with EB alone. Long-Evans females were androgenized in utero by a s.c. injection of 500 μg testosterone propionate or the oil control to pregnant dams on gestational day 18. Female offspring were OVX on postnatal day 80 and tested one week later in the unilevel 4-hole pacing chamber. Genital tissue was defeminized in PNAFs, and the lordosis quotient (LQ) and partial (i.e., hops/darts) and full solicitations were significantly lower, while defensive behaviors were higher, in PNAF females, relative to non-PNAF females regardless of the acute EB priming dose. However, repeated testing with EB alone (10 μg), or EB and P eliminated the differences between groups on LQ and hops/darts, indicating that the behavioral deficit can be overcome by sexual experience. These results suggest that PNAFs are not desensitized to EB, and despite disruptions in sexual differentiation of anatomical structures, the deficiency in sexual behavior in response to acute EB and P can be experientially overcome. PNAFs appear, however, to have a chronic deficit in the expression of full solicitations.

Introduction

The hormonal environment during the perinatal period organizes reproductive and neural tissue in such a way that steroid hormone administration in adulthood differentially activates sexually dimorphic sexual behaviors (Dunlap et al., 1978; Gerall and Ward, 1966; Phoenix et al., 1959; Rhees et al., 1997). The presence of biologically active aromatizable androgens during the critical perinatal period in rodents induces masculinization and defeminization, whereas the absence of androgens results in feminization. Behaviorally, a single injection of testosterone administered from gestational day (GD) 18 up to GD22 disrupts the expression of female-typical sexual behavior (i.e., lordosis, ear wiggling, hops/darts) in rats ovariectomized (OVX) in adulthood, when treated with doses of estradiol benzoate (EB) and progesterone (P) that are effective in untreated controls (Rhees et al., 1997). Similarly, sexual dimorphisms induced by early hormone exposure are seen anatomically. For example, males as well as perinatally androgenized females (exposed to testosterone from GD18 up to postnatal day 5) have a larger sexually dimorphic nucleus of the preoptic area (SDN-POA) (Anderson et al., 1985; Döhler et al., 1982; Gorski et al., 1978, 1980; Rhees et al., 1990a,b), and a longer anogenital distance (AGD) (Rhees et al., 1997) compared to untreated females.

Although the behavioral disruptions by perinatal hormone treatments are often considered permanent, earlier studies suggest that impaired female sexual behavior induced by prenatal androgens can be at least partially overcome by altering the hormonal priming conditions. Following gonadectomy in adulthood, perinatally androgenized female rats, as well as male rats (that are presumably fully androgenized), treated with estradiol followed by P display rates of lordosis similar to that observed in OVX control rats in adulthood, provided estradiol is administered in a pulsed fashion (Olster and Blaustein, 1988; Södersten, 1976; Södersten et al., 1983). In some cases, male rats are also capable of displaying P-facilitated appetitive sexual behaviors such as ear-wiggling (Södersten, 1976), although not more active forms such as hops and darts (Olster and Blaustein, 1988). Whether permanent deficits occur on other measures of appetitive sexual behaviors, such as full solicitations (McClintock, 1984), is not known.

Two critical hypothalamic regions that control female sexual behavior in a variety of species are the medial preoptic area (mPOA) and ventromedial hypothalamus (VMH). In adult female rats, estradiol binding to its receptors (ER) within the VMH induces lordosis, and promotes P receptor synthesis in both these regions (MacLusky and McEwen, 1978).

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Progesterone binding to its receptor in the VMH potentiates lordosis, and binding within the mPOA induces sexually appetitive behaviors (such as hops/darts, solicitations, and ear wiggles) (Beyer et al., 1997; Hoshina et al., 1994; Mani et al., 1994; Rubin and Barfield, 1983). Within these regions ER densities are sexually dimorphic in favor of females (Brown et al., 1990; Lauber et al., 1991a,b). This is mediated, at least in part, by aromatization of testosterone to estradiol during the perinatal period which organizes sexual differentiation of the brain and subsequent behavior (McCarthy, 2008; McCarthy et al., 2008). Perinatal exposure to estradiol methylates the ERα promoter region, associated with a down-regulation of ERα mRNA (DonCarlos et al., 1995; Kurian et al., 2010). As such, early androgen exposure permanently reduces ERα expression, and it has been suggested that females are permanently desensitized to the activational effects of estrogens (MacLusky et al., 1997). In support of this, the expression of estradiol-induced P receptors is attenuated in PNAFs, suggesting that neurochemical events triggered by estradiol are disrupted (Foecking et al., 2005).

Although these data suggest a reduced responsivity of perinatally androgenized female rats to estradiol, the degree of this insensitivity and whether it can be fully or partially rescued by subsequent hormonal treatment has not been fully explored. Thus in the first study described here we asked whether sexual behavior deficits induced in females by prenatal administration of testosterone propionate (TP) could be overcome, by increasing doses of an acute EB treatment administered 44 h prior to P. Next we tested whether those prenatally androgenized females (PNAFs) would display behavioral sensitization to chronic EB. Although, acute administration of EB alone only partially activates female sexual behaviors, repeated administration induces higher levels of sexual behaviors than the initial injection (Babcock et al., 1988; Beach and Orndoff, 1974; Blaustein et al., 1987; Clark and Roy, 1983; Gerall et al., 1973; Jones et al., 2013; Kow and Pfaff, 1975; Parsons et al., 1979; Whalen and Nakayama, 1965). For example, we recently reported that when treated every four days, both OVX and OVX-adrenalectomized sexually-experienced Long-Evans rats administered 10 μg EB display increasingly greater lordosis quotients (LQ) and sexually appetitive (hops/darts + solicitations) behaviors with each injection, reaching a plateau by the fourth test (Jones et al., 2013). EB doses below 3 μg, when repeatedly administered alone however, are insufficient to induce sexual behavior (Jones et al., 2013; Kow and Pfaff, 1975; Micevych et al., 2008; Sinchak and Micevych, 2001) if the female has access to the male on every test (Jones and Pfaus, 2014). Because PNAFs are less sensitive to the activational effects of EB on sexual behavior, in the second study described below, we investigated whether those females would express behavioral sensitization to repeated injections of EB, following sexual experience with EB and P. We hypothesized that if PNAFs were permanently desensitized to EB, as proposed by the organizational hypothesis, they would remain impaired to the activational effects of steroid hormone priming on sexual behaviors regardless of the EB dose administered, and fail to display behavioral sensitization to repeated EB treatments.

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, mating, and prenatal treatment procedures

All animals in this study were housed in colony rooms maintained at approximately 21 °C on a 12:12 light:dark schedule (lights off at 0800). Animals were given ad libitum access to food (Charles River, 5075) and tap water at all times, and environmental enrichment in the form of shredded paper was added to the homecage at each bi-weekly cage change. Home cages as well as the testing apparatus were lined with Betachip®. Adult males and females were housed in pairs in Plexiglass shoebox cages (48 × 25 × 20 cm) unless otherwise indicated.

Dams

Twelve females of the Long-Evans strain were used. Sexual receptivity was verified daily by placing the female in a unilevel 4-hole pacing chamber with a sexually vigorous male. Copulation in these chambers allows the smaller female to pace the rate of copulation by entering the males' side of the chamber, whereas the larger male is restrained to his side. Pacing the rate of sexual contact at her preferred interval is rewarding to the female, and increases the probability of impregnation (Erskine et al., 1989; Jenkins and Becker, 2003; Paredes and Vazquez, 1999). Those displaying proceptive hopping, darting and solicitations as well as lordosis in response to a mount were left to copulate for 1 h. The experimenter ensured that females received at least one ejaculation, and this day was considered GD0.

Impregnation was successful in 7 of the 12 time-mated females, verified by an increase in body weight accompanied by abdominal distention in the weeks following copulation. These females were randomly given a s.c. injection of either sesame oil (Oil, n = 3) or 500 μg of TP dissolved in 0.5 mL of sesame oil (TP, n = 4) on GD18, were housed individually, and provided with shredded paper for nest building. This TP dose disrupts genital morphology and the expression of female-typical sexual behaviors in adulthood (Rhees et al., 1997). As of GD20, twice a day (approximately 8 AM and 5 PM) a researcher verified whether the pregnant females had given birth. The day of birth was identified as postnatal day zero (PN0). Between PN21 and 23 the pups were weaned, sexed and housed in same-sex groups of 3 or 4 until they required additional space (approximately 2 months of age), when they were finally housed in pairs until the end of the experiment. Since males were not experimental subjects in the study, those from oil-treated mothers were distributed as stimulus animals in other studies, whereas those from TP-treated mothers were sacrificed under CO_2 gas following weaning.

Males

A group of Long-Evans male rats (~6 months of age) that had previously been used in unrelated studies in our laboratory were used for mating, and behavioral training and testing. They were sexually experienced in the unilevel 4-hole pacing chambers.

Four Long-Evans males (between 95 and 98 days of age) obtained from the same supplier and used in unrelated studies in our lab, were used as controls in the anogenital distance analyses.

Experimental females

Females born from dams treated on GD18 were used as experimental animals. On GD80 females were OVX. On PN95 the anogenital region was photographed and anogenital distance was measured using a caliper.

Ovariectomy

Anesthesia was induced with a 4:3 mixture of ketamine hydrochloride (50 mg/mL; Ketaset®, Wyeth Canada) and xylazine hydrochloride (4 mg/mL; Rompum®, Bayer Healthcare) injected i.p (1 mL/kg). An ocular ointment was applied (Natural Tears, Alcon), and when unresponsive to a foot pinch, bilateral OVX was performed via a single lumbar incision. Animals were numbered by ear-punch and post-operative care was given with s.c. injections of Flunixin meglumine 2.5 mg/kg (Banamine®, an anti-inflammatory, analgesic, and antipyretic) and 5 mg/kg Enrofloxacin (Baytril®, an antibacterial), followed by 3 mL of saline administered s.c., and polysporin was applied to the incision site. Animals were given one-week post-operative recovery prior to testing.