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The role of neonatal NMDA receptor activation in defeminization and masculinization of sex behavior in the rat

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ABSTRACT

Normal development of the male rat brain involves two distinct processes, masculinization and defeminization, that occur during a critical period of brain sexual differentiation. Masculinization allows for the capacity to express male sex behavior in adulthood, and defeminization eliminates or suppresses the capacity to express female sex behavior in adulthood. Despite being separate processes, both masculinization and defeminization are induced by neonatal estradiol exposure. Though the mechanisms underlying estradiol-mediated masculinization of behavior during development have been identified, the mechanisms underlying defeminization are still unknown. We sought to determine whether neonatal activation of glutamate NMDA receptors is a necessary component of estradiol-induced defeminization of behavior. We report here that antagonizing glutamate receptors during the critical period of sexual differentiation blocks estradiol-induced defeminization but not masculinization of behavior in adulthood. However, enhancing NMDA receptor activation during the same critical period mimics estradiol to permanently induce both defeminization and masculinization of sexual behavior.

Introduction

Sex differences in the brain underlie sex differences in behavior, and this association is best characterized for rat sexual behavior. Sex differences in brain and behavior are determined during a sensitive period of development, with the hormone estradiol being critically important. In the male rat, the embryonic and neonatal testes produce testosterone that is locally aromatized to estradiol in select nuclei of the brain. In rats and mice, the critical period for sexual differentiation begins before birth and ends approximately 10days after birth. Within that time, treatment of females with exogenous estradiol will mimic the effect of endogenous estradiol in the male, to permanently change the brain and behavior in adulthood (Schwarz and McCarthy, 2008).

The normal development of the male brain requires completion of two distinct processes: masculinization and defeminization (Baum, 1979). Masculinization is the organization of a neural substrate permissive to the expression of male sexual behavior. Defeminization is the loss of capacity to respond to the activational effects of estradiol and progesterone to induce female sex behavior. Both processes oppose the process of feminization that induces the capacity to respond to estradiol and progesterone in adulthood with lordosis, or female sexual receptivity. Feminization occurs in the absence of critical levels of neuronal estradiol during the neonatal critical period (Baum, 1979; Nordeen and Yahr, 1983). Advances are being made in understanding the mechanisms by which steroids induce masculinization of the brain and behavior, but little is know regarding the concurrent process of defeminization.

The preoptic area (POA) is a brain region necessary for male sex behavior and the mediobasal hypothalamus (MBH) is a brain region necessary for female sex behavior. Both regions are key targets of estradiol in development and in adulthood. In the neonatal POA and the MBH, males have two–three times more dendritic spines and spine synapses than females (Amateau and McCarthy, 2002; Matsumoto and Arai, 1980; 1986; Raisman and Field, 1973; Raisman, 1974; Todd et al., 2005; 2007), which are induced by estradiol during the critical period (Amateau and McCarthy, 2002; Todd et al., 2005; 2007). Estradiol induces dendritic spine formation in the POA by increasing the production of prostaglandin E2 (PGE2) via up-regulation of its synthesizing enzyme cyclooxygenase-2 (COX-2) (Amateau and McCarthy, 2002). Treatment of females with PGE2 mimics the effect of estradiol to increase dendritic spines on neurons in the POA (Amateau and McCarthy, 2002; 2004), but does not increase the number of dendritic spines on neurons in the neighboring MBH (Todd et al., 2005). Instead, estradiol increases dendritic spines in the developing MBH by enhancing glutamate release from presynaptic terminals to increase the activation of AMPA and NMDA glutamate receptors on postsynaptic hypothalamic neurons (Todd et al., 2007;

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Schwarz et al., 2008). Conversely, while activation of NMDA receptors is necessary and sufficient for estradiol to increase dendritic spines in the MBH during the critical period of development (Schwarz et al., 2008), activation of NMDA receptors is not necessary for estradiol or PGE2 to increase dendritic spines in the developing POA (Amateau and McCarthy, 2002).

We have previously determined that treatment of newborn female rat pups with PGE2 selectively induces complete masculinization of sex behavior in adulthood. Conversely, blocking estradiol-induced production of PGE2 using a COX-2 inhibitor, prevents masculinization of sex behavior (Amateau and McCarthy, 2002; 2004). However, these manipulations have no effect on the expression of female sex behavior, leaving the process of estradiol-induced defeminization intact (Todd et al., 2005). Taken together, these results lead to two conclusions; 1) estradiol-induced sex differences in neuronal morphology of the POA are not the site of estradiol-induced defeminization of behavior, 2) estradiol-induced sex differences in neuronal morphology of the MBH are not the underlying site of estradiol-induced masculinization of behavior. We therefore hypothesized that activation of NMDA glutamate receptors during the critical period of sexual differentiation is necessary and sufficient for the defeminization of behavior in adulthood. To test this hypothesis, we blocked the activation of NMDA receptors using the antagonist, AP5, in the presence of estradiol during the critical period of sexual differentiation, and tested animals in adulthood for the expression of female sex behavior. Additionally, we treated female pups neonatally with NMDA and tested animals in adulthood for the expression of female sex behavior. We determined that antagonizing NMDA receptors neonatally blocked estradiol-induced defeminization, while having no effect on estradiol-induced masculinization. However, direct activation of NMDA receptors neonatally induced defeminization, but also induced masculinization of the brain and behavior.

Materials and methods

Animals

All animal experiments were approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee. Female Sprague Dawley rats (Harlan Laboratories; Frederick, MD), maintained on a reverse 12 h light/dark cycle and provided *ad libitum* food (Harlan 2018, 8% rat diet) and water; were mated in our animal facility and pregnancy confirmed by presence of sperm in a vaginal smear. Pregnant females were isolated and allowed to deliver normally. Cages were checked regularly for presence of pups to determine the time and day of birth, designated postnatal day 0 (PN0).

Neonatal treatments

Experiment 1: Effect of neonatal NMDA receptor antagonist on estradiol-induced defeminization of sex behavior in adulthood

On the day of birth (PN0), pups were collected and separated into the following groups (see Table 1): 1) females treated with oil or saline (vehicle, $N=7$), 2) females treated with 100 μg estradiol benzoate ($N=6$) (Amateau et al., 2004), 3) females treated with estradiol and 10μg AP5, the NMDA receptor antagonist ($N=8$), 4) females treated with AP5 alone ($N=3$). The doses of estradiol and AP5 were based on those previously used to investigate estradiol-induced dendritic spines in the developing POA (Amateau and McCarthy, 2002). Animals from each treatment group were evenly represented across 3–4 litters (1–3 females per litter per treatment group depending on litter size). All pups were housed with their original mother and littermates through the duration of the experiment. Males were not used in these experiments and were sacrificed on the day of birth to cull litter sizes to 6–8 pups.

Table 1
Treatment groups and behavioral predictions

Neonatal treatment	Female sex behavior: Defeminization = NO	Male sex behavior: Masculinization = YES
Female + Vehicle	YES/YES	NO/NO
Female + E_2	NO/NO	YES/YES
Female + E_2 + AP5	YES/YES	YES/YES
Female + AP5	YES/YES	NO/NO
Female + NMDA	NO/NO	NO/YES[a]

[a]Neonatal treatment groups and the predicted adult behavioral outcomes are listed for Experiments 1 and 2. Female sexual behavior was tested following appropriate hormonal priming and placement of the test animal with a sexually experienced male. Male sexual behavior was tested in the same animals two weeks after s.c. implantation of a testosterone releasing silastic capsule and placement with a sexually receptive female rat. Defeminization of behavior is manifested as very low LQ scores. Masculinization of behavior is manifested as high levels of mounting and thrusting with a short latency. All observations were consistent with our predictions with the exception of the effects of NMDA treatment on masculinization (see highlighted box).

Pups were tattooed with India Ink for individual identification and injected subcutaneously once a day, everyday from PN0 inclusive of PN2 for a total of 3 injections of their respective treatment. Animals were weaned from the dam on PN21 and housed 2–3 per cage.

Experiment 2: Effect of neonatal NMDA receptor activation on the defeminization of sex behavior in adulthood

On the day of birth, pups were separated into the following groups (see Table 1); 1) females treated with oil or saline (vehicle $N=9$), 2) females treated with 100 μg estradiol in 0.1 cm^3 oil ($N=9$), 3) females treated with 10 μg NMDA in 0.05 cm^3 saline ($N=9$). Pups were tattooed for identification and given these treatments once a day, everyday from PN0 till PN2, and then weaned and housed in the same manner as for Experiment 1.

Behavioral testing

On PN40 all animals were ovariectomized under Ketamine/Acepromazine anesthesia and allowed to recover for 10 days. All animals were coded with markings on the tail to blind the experimenter to the treatment groups and were injected with 10 μg estradiol benzoate in 0.1 cm^3 sesame oil subcutaneously 48 h and 24 h prior, and 500 μg progesterone in 0.2 cm^3 sesame oil 4h prior to female sexual behavioral testing. This hormone dosing regime reliably induces female sexual receptivity (Todd et al., 2005). Female sex behavior was quantified as; 1) lordosis quotient (LQ), which is the number of lordoses per 10 mounts × 100, and 2) lordosis score (LS), a rating from 0 (no response) to 3 (complete dorsiflexion of the spine in the lordosis posture) of the female to a mount in the presence of a sexually experienced male until 10 mounts were received, and 3) the number of proceptive events expressed, including hopping, darting and ear-wiggling. A sequential expression of such behaviors was labeled as one event.

One week following female sexual behavior testing (PN59), a 30 mm silastic capsule (1.57 mm id, 3.18 mm od) filled with crystalline testosterone was subcutaneously implanted at the nape of the neck to standardize circulating hormone concentrations to that of normal adult males (Amateau and McCarthy, 2004). Two weeks later, testing for male-typical sexual behavior began and was conducted once a week for three weeks for a total of three trials. All subject animals were observed over a 15 min period for the display of male-typical sexual behavior when placed in an arena with a hormonally-induced sexually receptive stimulus female. Parameters of male sex behavior quantified were the number of mounts, number of mounts with thrusts, latency to first mount, and the latency to first mount with thrusts; during a 15 minute testing period. Behaviors were characterized as previously described (Todd et al., 2005). Mounts were