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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 10 days 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

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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 known 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; Matsumoto and Arai, 1986; Raisman and Field, 1973; Raisman, 1974; Todd et al, 2005; Todd et al, 2007), which are induced by estradiol during the critical period (Amateau and McCarthy, 2002; Todd et al, 2005; Todd et al, 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; Amateau and McCarthy, 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, 200; 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; Amateau and McCarthy, 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 hr 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 I): 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 10 μg 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.

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 I): 1) females treated with oil or saline (vehicle $N = 9$), 2) females treated with 100 μg estradiol in 0.1 cc oil ($N = 9$), 3) females treated with 10 μg NMDA in 0.05 cc 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 cc sesame oil subcutaneously 48 hours and 24 hours prior, and 500 μg progesterone in 0.2 cc sesame oil 4 hours 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 $\times 100$, 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 distinguished as the placement of the subject animal's forelimbs on the stimulus female's flanks from a position behind the stimulus female. Mount with thrusts behavior was distinguished as a mount followed by rhythmic thrusting movements and a rapid backward dismounting action.

Data generated during female-typical sexual behavioral analyses were analyzed by one-way analysis of variance (ANOVA). Male-typical sexual behavior data were averaged across three trials and analyzed by ANOVA. All ANOVAs were followed by Tukey's post-hoc comparisons to determine differences between groups. All statistical significance was set at $\alpha = 0.05$.

Results

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

Female Sexual Behavior—There was an overall effect of neonatal treatment on mean LQ, (Lordosis Quotient: $F_{3,20} = 7.34$, $p = 0.002$). Control females exhibited a high level of sexual receptivity, with LQ values close to 80%. As predicted, treatment of neonatal females with estradiol completely defeminized sexual behavior in adulthood as these animals exhibited significantly lower levels of female sex behavior in the presence of a sexually experienced male, with an average LQ ~ 20%, compared to vehicle treated females and females treated with AP5 alone ($p = 0.001$ and $p = 0.0007$ respectively). Treatment of neonatal females with AP5, to block activation of the NMDA glutamate receptor, in the presence of estradiol significantly blocked estradiol-induced defeminization of behavior, as these animals had significantly higher LQs than females treated with estradiol alone ($p = 0.028$; Figure 1A) and these LQs were not significantly different from females treated neonatally with vehicle ($p = 0.109$). Treatment of females with AP5 alone neonatally had no effect on the expression of female sex behavior, such that these females were highly sexually receptive and did not differ from vehicle treated controls ($p = 0.32$). Despite differences in the frequency of lordosis responding, there was no effect of neonatal treatment on the strength of the lordotic response, as indicated by the Lordosis Score ($F_{3,20} = 0.91$; $p = 0.452$; Table II), nor were there any differences in the expression of proceptive behaviors; including hopping, darting and ear-wiggling between treatment groups ($F_{3,20} = 0.82$; $p = 0.495$; Table II). Thus, we conclude that antagonism of NMDA receptors during development blocked estradiol-induced defeminization of behavior in adulthood.

Male Sexual Behavior—In adulthood, we found an overall effect of treatment on the number of mounts ($F_{3,20} = 12.23$; $p = 0.0001$, Figure 1B) and the number of mounts with thrusting ($F_{3,20} = 5.12$; $p = 0.008$, Table III) during the course of the behavioral testing. Specifically, females treated neonatally with estradiol also performed significantly more mounts and mounts with thrusts than females treated with vehicle neonatally ($p < 0.001$ and $p = 0.0042$ respectively). We also found an overall effect of neonatal treatment on the latency to mount ($F_{3,20} = 5.97$; $p = 0.004$) and the first mount with thrusting behavior ($F_{3,20} = 22.94$; $p < 0.001$). Females treated with estradiol neonatally exhibited significantly shorter latencies to the first mount ($p = 0.006$) and to the first mount with thrusts than vehicle-treated females ($p < 0.0001$, Table III). In this study neonatal treatment of females with estradiol induced behavioral masculinization similar to that seen in normal males in previous studies from this laboratory (Todd et al, 2005). Animals treated with both estradiol and the NMDA receptor antagonist, AP5, also exhibited shorter latencies to first mount ($p = 0.0063$), shorter latencies to first mount with thrusts ($p < 0.001$), more mounts ($p = 0.005$) and more mounts with thrusts ($p = 0.008$)

than vehicle-treated females. However, the level of these behaviors in animals treated with both estradiol and AP5 were not significantly different from females treated with estradiol alone ($p = 0.36$ latency to mount; $p = 0.12$ latency to mount with thrusts; $p = 0.18$ number of mounts; and $p = 0.60$ number of mounts with thrusts). Females treated neonatally with vehicle or AP5 alone exhibited only low levels of male sex behavior, as determined by number of mounts, in adulthood ($p = 0.001$ and $p = 0.0015$ compared to estradiol and estradiol + AP5 treated females, respectively). Thus, antagonism of NMDA receptors during the critical period for sexual differentiation blocked estradiol-induced defeminization but had no effect on estradiol-induced masculinization of behavior.

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

Female Sexual Behavior—In a separate experiment, we again found an overall effect of neonatal treatment on mean LQ ($F_{2,24} = 12.2$; $p = 0.0002$; Figure 2A). Specifically, females treated neonatally with vehicle showed high levels of sexual receptivity, with a mean LQ of approximately 80%. Females treated neonatally with estradiol had significantly lower levels of sexual receptivity in adulthood compared to vehicle treated females ($p = 0.0001$), indicating their brains were defeminized. Females treated neonatally with NMDA also displayed significantly lower levels of female sexual receptivity in adulthood, with a mean LQ of approximately 30%, indicating that enhanced NMDA receptor activation during the critical period of sexual differentiation was sufficient to induce defeminization of behavior ($p = 0.0006$; compared to vehicle treated females, Figure 2A). As seen in the previous experiment, there were no significant differences between treatment groups in the Lordosis Score, although there was a strong trend which is likely due to the lower LS in the estradiol treated females ($F_{2,24} = 2.74$; $p = 0.08$; Table II). However, there was an overall effect of neonatal treatment on the number of proceptive events expressed during female sexual behavioral testing in this experiment ($F_{2,24} = 5.90$; $p = 0.008$; Table II). Specifically, females treated neonatally with vehicle showed high levels of the proceptive behaviors, hopping and darting; compared to females treated with estradiol neonatally ($p = 0.006$), and females treated with NMDA neonatally also showed high levels of proceptive behaviors ($p = 0.006$ compared to estradiol-treated females) despite their low level of receptivity. These data suggest the expression of lordosis and the expression of proceptive behaviors may be defeminized via separate developmental processes and NMDA receptor activation in the developing hypothalamus is exclusively a mechanism involved in estradiol-induced defeminization of female sexual receptivity, not proceptivity.

Male Sexual Behavior—There was an overall effect of neonatal treatment on the number of mounts ($F_{2,25} = 17.46$; $p = 0.0001$ Figure 2B) and mounts with thrusts ($F_{2,25} = 12.87$; $p < 0.001$ Table III) displayed during the course of behavioral testing. Specifically, females treated neonatally with estradiol performed significantly more mounts and mounts with thrusts than vehicle-treated females ($p = 0.0002$ and $p = 0.02$ respectively, Figure 2B and Table III). In addition, females treated neonatally with NMDA performed significantly more mounts than females treated with vehicle ($p < 0.001$), at levels that were not significantly different from estradiol-treated females ($p = 0.20$). However, females treated neonatally with NMDA showed significantly fewer mounts with thrusts than females treated with estradiol neonatally ($p = 0.017$). There was also an overall effect of neonatal treatment on the latency to first mount ($F_{2,25} = 9.18$; $p = 0.001$) and the latency to first mount with thrusts ($F_{2,25} = 10.09$; $p < 0.001$, Table III) during adult male sexual behavior testing. Females treated neonatally with vehicle showed little expression of male sex behavior, indicating no masculinization of the brain. Females treated with estradiol neonatally exhibited significantly shorter latencies to the first mount and to the first mount with thrusts than vehicle-treated females ($p = 0.0004$ and $p = 0.0003$ respectively, Table III). Females treated with NMDA exhibited latencies to first mount

and mount with thrusts that were not significantly different than estradiol-treated females (mount latency, $p = 0.396$; mount + thrust latency, $p = 0.44$). Together, these data suggest that treatment of females with NMDA neonatally was sufficient to induce motivated male sex behavior.

Correlation Between Measures of Female and Male Sex Behavior in the Same Animals—The sequential analysis of female and male sex behavior in the same animals allowed for the opportunity to determine whether there was a correlation between the probability of lordosis and the mount frequency expressed in each individual animal. There was a significant negative correlation between the degree to which male and female sex behavior was expressed ($R^2 = 0.52$, $Z_{51} = -6.37$, $p < 0.0001$; Figure 3). Animals with a low LQ were more likely to exhibit a high number of male mounts and vice versa. This was true whether the animals were treated with estradiol, AP5, NMDA or vehicle. These results suggest that although the processes of masculinization and defeminization have separate underlying mechanisms, their induction may be interconnected. Therefore, as defeminization is initiated, via enhanced NMDA receptor activation, masculinization might also be initiated in an estradiol-independent manner.

Discussion

The concept that masculinization and defeminization are two separate processes is not new (Baum, 1979; Feder and Whalen, 1965; Kudwa et al, 2005; Reinisch et al, 1991), however distinguishing them has been difficult as both processes are mediated by the same steroid, estradiol. Recent advances have been made identifying the underlying cellular signals of estradiol that mediate each process. There are two major isoforms of the estrogen receptor (ER), ER alpha and ER beta. ER alpha is the predominant isoform found in the developing hypothalamus and in other brain regions known to be sexually dimorphic (Shughrue et al, 1997). Male mice lacking a functional ER-alpha show low levels of male sex behavior in adulthood, implicating this receptor isoform in the process of masculinization (Wersinger et al, 1997). Conversely, the process of estradiol-mediated defeminization appears to be induced by activation of ER-beta as male mice lacking a functional ER-beta show significantly higher levels of lordosis behavior compared with wild type males, but no differences in the expression of male sex behavior (Ogawa et al, 1999; Kudwa et al, 2005). These data indicate that ER isoform may play a role in distinguishing the processes of masculinization and defeminization. However, in the absence of a functioning ER throughout development and adulthood, it is difficult to distinguish the role of each isoform exclusively during the process of sexual differentiation.

In the current experiment we blocked an underlying mechanism of estradiol action at its receptor in the developing brain, i.e. glutamate NMDA receptor activation, exclusively during the critical period of sexual differentiation. In doing so, we prevented the process of estradiol-induced defeminization. Specifically, animals treated with an NMDA receptor antagonist in the presence of estradiol during the critical period for sexual differentiation showed adult levels of female sex behavior that were not significantly different from normal females upon exposure to adult female circulating hormones. These data indicate that estradiol-induced defeminization requires activation of NMDA glutamate receptors in the brain.

We recently reported that NMDA receptor activation is a necessary component of estradiol-induced sex differences in neuronal morphology in the developing mediobasal hypothalamus, and the ventromedial nucleus in particular (Schwarz et al, 2008). Dendrites of the neonatal male hypothalamus have more branches and more dendritic spines than females (Todd et al, 2007). Estradiol establishes these sex differences by enhancing glutamate release from hypothalamic terminals, activating postsynaptic NMDA glutamate receptors. Blocking the

NMDA receptor in the neonatal brain blocks the estradiol-induced formation of dendritic spines in the hypothalamus (Schwarz et al, 2008). The results of the present experiments demonstrate that blocking NMDA glutamate receptors during neonatal development, which blocks estradiol-induced changes in neuronal morphology (Schwarz et al, 2008), increases the expression of female sexual behavior in adulthood as measured by the lordosis quotient, but has no effect on the expression of male sexual behavior. Thus sex differences in neuronal morphology induced by estradiol-enhanced glutamatergic activation of NMDA receptors may be the underlying structural basis for behavioral defeminization into adulthood. This notion is further supported by the observation that enhanced NMDA receptor activation alone is sufficient to mimic estradiol and induce a sex difference in the neuronal morphology of the neonatal hypothalamus (Schwarz et al, 2008) and defeminize behavior in adulthood.

In the adult, glutamate receptor activation in the hypothalamus inhibits the expression of lordosis (Georgescu and Pfau, 2006a; Georgescu and Pfau, 2006b; McCarthy et al, 1991). By extension, one might predict that estradiol-induced increases in dendritic spines on male hypothalamic neurons during development inhibit lordosis in adulthood as a result of permanently increased glutamatergic transmission in this region. The expression of female sex behavior requires a combination of precisely timed inputs from activational hormones, vomeronasal stimulation and somatosensory stimulation. These inputs converge at the level of the MBH before the signal to lordose is sent down to the spinal cord (Kow and Pfaff, 1981). In the current study, each experimental female, defeminized or not, received the appropriate activational hormones as well as the vomeronasal and somatosensory input from the male throughout the course of behavioral testing. However, permanent increases in dendritic spines due to neonatal estradiol, and therefore NMDA receptor activation, would maintain increased glutamatergic transmission and therefore inhibit the expression of lordosis despite normal detection of the salient cues.

Alternatively, neonatal estradiol may affect the responsiveness of individual hypothalamic neurons to any one of the relevant cues necessary for female sex behavior, be they hormonal, olfactory, or tactile. Treatment of normal females with estradiol in adulthood induces formation of dendritic spines on hypothalamic neurons (Calizo and Flanagan-Cato, 2000; Cohen and Pfaff, 1992; Flanagan-Cato et al, 2001), but the same treatment of males has no effect (Lewis et al, 1995) indicating that neurons in the hypothalamus of an adult male, or a defeminized female, are unresponsive to the activational effects of hormones relevant to female sex behavior. Therefore, sex differences in hypothalamic dendritic spine morphology, induced by activation of NMDA receptors during development, may induce a permanent sex difference in the responsiveness of the neurons to activational hormones, as opposed to an actual change in the circuitry underlying the behavior between the sexes. How adult neuronal plasticity in response to steroids is constrained by early steroid effects is a poorly understood and under-investigated area.

An unexpected observation in these experiments was that NMDA treatment of neonatal females induced significant masculinization of behavior in addition to defeminization of behavior. Antagonizing the NMDA receptor with AP5 blocked the defeminizing effect of estradiol, but the high variability in this treatment group attracted our interest and led us to perform an analysis to correlate the expression of female sexual receptivity and male sex behavior from the same animals. We discovered that animals that were more defeminized, or expressed lower levels of female sexual receptivity, were also more masculinized, expressing greater levels of male sex behavior; and vice versa. This was true whether the animals were treated with estradiol, AP5 or NMDA. The significant inverse correlation between the levels of male versus female sexual behavior suggests the initiation of masculinization and defeminization are interconnected. This would seem in direct contradiction to previous work from our lab, which found that treatment of females with PGE2 neonatally induces complete masculinization of

the brain and behavior, while having no effect on defeminization (Amateau and McCarthy, 2004; Todd et al, 2005). However, we did not assess the relationship between male and female sex behavior in those animals, and the degree of masculinization was so complete that there may not have been sufficient variability to discern a meaningful relationship.

Based on the current and previous observations, we speculate that the induction of behavioral masculinization might occur independent of behavioral defeminization, while the process of defeminization is mechanistically linked to masculinization in order to prevent the development of an animal that expresses no sexual behavior in adulthood. In other words, masculinization induced by PGE2 proceeds independently of defeminization, but when defeminization is initiated by NMDA receptor activation it secondarily initiates a redundant pathway to masculinization, independent of estradiol. This end could be achieved by the global activation of NMDA receptors neonatally, as occurred in these experiments, thereby inducing changes in the neighboring POA via reciprocal connections or via direct actions in the POA, such as inducing the synthesis of PGE2. Although NMDA receptor activation is not necessary for estradiol-induced changes in neuronal morphology of the POA (Amateau and McCarthy, 2002), it may be sufficient, or NMDA receptor activation may be necessary for other unexplored parameters of masculinization.

In conclusion, region specific mechanisms of estradiol action in development coordinate to achieve the specific goal of sexual differentiation of the male brain and coordinate the concurrent processes of masculinization and defeminization. An intrinsic consequence of these diverse mechanisms of estradiol action is greater individual variability in behavioral phenotype than would be achieved by a single unified mechanism of estradiol-induced masculinization and defeminization.

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Figure 1. NMDA receptor activation is necessary for estradiol-induced defeminization not masculinization of behavior

A. Neonatal treatment with estradiol significantly decreased the LQ compared to all other groups (ANOVA, $*p < 0.05$), including females treated with estradiol + AP5, an NMDA receptor antagonist.

B. Neonatal treatment with estradiol induced masculinization of behavior as determined by the total number of mounts and pretreatment with AP5 and estradiol had no effect on the expression of male sex behavior (ANOVA, $*p < 0.05$ compared to Vehicle or AP5 alone). Data represent the mean $\pm$ SEM (Vehicle $n = 7$, Estradiol $n = 6$, Estradiol + AP5 $n = 8$, AP5 $n = 3$).

Figure 2. Activation of NMDA receptors in neonatal females induces defeminization and masculinization of behavior

A. Neonatal treatment with estradiol or NMDA significantly reduced the LQ (ANOVA, * $p < 0.05$ compared to Vehicle).

B. Neonatal treatment with either estradiol or NMDA significantly equally increased the number of mounts exhibited during male sexual behavior tests (ANOVA, * $p < 0.05$ compared to Vehicle). Data represent the mean $\pm$ SEM (Vehicle $n = 9$, Estradiol $n = 9$, NMDA $n = 9$).

Figure 3. Masculinization and defeminization of behavior are inversely correlated

All animals, regardless of treatment, were tested for both male and female sexual behavior. Correlation analysis of female sex behavior, or Lordosis Quotient, and the expression of male sex behavior, or number of mounts, indicates a significant inverse relationship between the two parameters in the same animals regardless of treatment ($R^2 = 0.52$, $Z_{51} = -6.37$, $p < 0.0001$), supporting the notion that masculinization and defeminization of sexual behavior are separate yet interconnected processes.

Table I**Treatment groups and behavioral predictions**

Table I. 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 2 weeks after s.c. implantation of a testosterone releasing silastic capsule and placement with a sexually receptive female rat. Defeminization of behavior is manifest as very low LQ scores. Masculinization of behavior is manifest 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)

Neonatal Treatment	Female Sex Behavior: Deferminization = NO	Mate 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*

Table II**Adult female sexual behavior in neonatally treated females**

Table II. Experiment 1. There was no significant effect of neonatal treatment on the Lordosis Score or the number of Preceptive Events expressed during female sex behavior (ANOVA), despite the significant effects on LQ (Figure 2). Values represent the mean $\pm$ SEM (Vehicle n = 7, Estradiol n = 6, Estradiol + AP5 n = 8, AP5 n = 3).

Experiment 2. There was no effect of neonatal treatment on the Lordosis Score (ANOVA) There was, however, an overall effect on the number of preceptive events (ANOVA; $p < 0.05$), while estradiol significantly decreased the number of preceptive events, neonatal treatment with NMDA did not impair proceptivity (* $p < 0.05$ compared to Vehicle and NMDA). Values represent the mean $\pm$ SEM (Vehicle n = 9, Estradiol n = 9, NMDA n = 9)

Experiment 1		
Neonatal Treatment	Lordosis Score (mean $\pm$ SEM)	No. of Preceptive Events (mean $\pm$ SEM)
Vehicle	2.79 $\pm$ 0.082	5.28 $\pm$ 1.30
Estradiol	2.53 $\pm$ 0.184	2.5 $\pm$ 1.43
Estradiol + AP5	2.25 $\pm$ 0.393	3.12 $\pm$ 2.1
AP5 alone	2.72 $\pm$ 0.147	3.0 $\pm$ 1.0
Experiment 2		
Neonatal Treatment	Lordosis Score (mean $\pm$ SEM)	No. of Preceptive Events (mean $\pm$ SEM)
Vehicle	2.55 $\pm$ 0.14	5.5 $\pm$ 1.84
Estradiol	1.71 $\pm$ 0.34	1.5 $\pm$ 0.53*
NMDA	2.02 $\pm$ 0.37	7.25 $\pm$ 2.28

Table III**Adult male sex behavior in neonatally treated females**

Table III. Experiment 1. Neonatal treatment with estradiol or estradiol plus AP5 induced masculinization of behavior as indicated by a greater number of mounts with thrusts. Neonatal estradiol treatment, with or without AP5 also significantly reduced the latency to mount and latency to mount with thrusts (ANOVA, * $p < 0.05$ compared to either Vehicle or AP5 alone-treated females for both measures). Values represent the mean $\pm$ SEM (Vehicle $n = 7$, Estradiol $n = 6$, Estradiol + AP5 $n = 8$, AP5 $n = 3$).

Experiment 2. Neonatal treatment with estradiol or NMDA induced masculinization of behavior as indicated by a greater number of mounts with thrusts (ANOVA). NMDA treatment significantly increased the number of mounts with thrusting, though significantly less than levels seen with neonatal estradiol treatment ($\# p < 0.05$ from Vehicle and from Estradiol). Neonatal estradiol and NMDA treatment significantly decreased the latency to mount and the latency to mount with thrusting (ANOVA, * $p < 0.05$ compared to Vehicle), Values represent the mean $\pm$ SEM (Vehicle $n = 9$, Estradiol $n = 10$, NMDA $n = 9$)

Experiment 1			
Neonatal Treatment	Total No. of Mounts with Thrusts (mean $\pm$ SEM)	Latency to Mount (sec.) (mean $\pm$ SEM)	Latency to Mount with Thrusts (sec.) (mean $\pm$ SEM)
Vehicle	0.0 $\pm$ 0.0	586.42 $\pm$ 90.89	900.0 $\pm$ 0
Estradiol	6.66 $\pm$ 1.38*	111.16 $\pm$ 37.89*	111.0 $\pm$ 25.64*
Estradiol + AP5	5.62 $\pm$ 1.97*	225.87 $\pm$ 103.04*	279.0 $\pm$ 103.54*
AP5 alone	0.33 $\pm$ 0.13	512.33 $\pm$ 96.81	750.66 $\pm$ 149.33
Experiment 2			
Neonatal Treatment	Total No. of Mounts with Thrusts (mean $\pm$ SEM)	Latency to Mount (sec.) (mean $\pm$ SEM)	Latency to Mount with Thrusts (sec.) (mean $\pm$ SEM)
Vehicle	1.44 $\pm$ 0.86	441.33 $\pm$ 121.88	540.00 $\pm$ 142.0
Estradiol	12.1 $\pm$ 1.79*	41.4 $\pm$ 6.84*	55.88 $\pm$ 7.59*
NMDA	6.77 $\pm$ 1.57*	125.44 $\pm$ 26.29*	143.11 $\pm$ 27.13*