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Behavioural Brain Research 167 (2006) 197–204
BEHAVIOURAL BRAIN RESEARCH
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Research report

Genotype differences in behavior and tyrosine hydroxylase expression between wild-type and progesterone receptor knockout mice

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Received 22 March 2005; received in revised form 13 July 2005; accepted 13 July 2005

Abstract

Progesterone receptor (PR) activation can modulate the expression of male sexual behavior, both acutely in adulthood as well as during development, through long lasting effects on neural differentiation. One mechanism by which PR activation may affect behavior, during either epoch of life, is through alterations of the dopaminergic system. We investigated the effects of PR deletion on the sensitivity of sexual behavior to dopamine antagonism in male wild-type (WT) and progesterone receptor knockout (PRKO) mice and found that WT mice were more behaviorally sensitive to the effects of dopamine D1 receptor blockade. There were also genotype differences in tyrosine hydroxylase-immunoreactivity (TH-ir) in the substantia nigra *pars compacta* (SNc) and the ventral tegmental area (VTA) as well as genotype differences in how TH expression changed in response to social and sexual experience. In particular, in the VTA, sexually experienced PRKO mice had significantly more cells expressing TH than sexually experienced WT mice. In the SNc, experienced PRKO males had significantly more cells expressing TH than naive PRKO males. Thus, it appears that PR deletion affects the display of sexual behavior and its modulation by dopamine, as well as the differentiation of dopaminergic cells and the plasticity of those cells in response to social environment and behavioral experience.

Keywords: Progesterone receptor; Dopaminergic system; Genotype difference

1. Introduction

Progesterone has been shown to modulate the display of sexual behavior in adult lizards and rats [24,25,51,56,57]. In addition, administration of a progesterone receptor (PR) antagonist to neonatal males diminishes masculine sexual behavior in adulthood [26]. Exogenous progesterone affects cellular morphology and differentiation during development [41,42] and may affect sexual differentiation of the brain. For example, in the anteroventral periventricular nucleus and medial preoptic nucleus male rats express higher levels of progesterone receptor perinatally than do females, and PR has been hypothesized to be involved in the sexual differentiation of these nuclei [36,37]. Male progesterone receptor knockout (PRKO) mice mount less frequently during their first sexual encounter than do male wild-type (WT) mice [35]. In addition, sexually experienced WT males continue to copulate after castration, while experienced PRKO males do not [35], which may indicate a difference in neural plasticity between the genotypes. These behavioral differences between PRKO and WT mice could be due to either the absence of PR during development or adulthood.

Dopamine also affects the display of courtship and copulatory behaviors in a number of taxa. Dopamine release into the POA, nucleus accumbens, and striatum increases during copulation in rats [11,19], while injections of dopamine agonists and antagonists facilitate and inhibit the display of sexual behaviors in mammals [20], birds [3,8], and reptiles [54,55]. In addition, the number of dopamine producing cells in the substantia nigra pars compacta (SNc) has been correlated with the propensity to display male courtship behavior in two species of lizards [52,53]. In mice PR is expressed perinatally in the SNc and the ventral tegmental area (VTA) [5] and the peak of PR expression in these nuclei is coincident with increases in the number and activity of tyrosine hydroxylase-immunoreactive (TH-ir) neu-

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doi:10.1016/j.bbr.2005.07.035

rons and the formation of functional contacts between midbrain dopaminergic cells and cells in the striatum [2]. The SNc and VTA have been implicated in motor and motivational aspects of behavior, including courtship and sexual behavior [20,45,46]. Thus, interactions between PR and catecholaminergic systems, either during development or in adulthood, may underlie genotype differences in behavior and plasticity between PRKO and WT mice.

We investigated whether behavioral differences between WT and PRKO males were associated with differences in dopaminergic systems. We first tested the sexual behavior of male WT and PRKO mice in response to a dopamine D1 receptor antagonist and found that the percent of WT males copulating was significantly decreased by treatment with the antagonist, while the behavior of PRKO males was not significantly affected. To begin to investigate the neural correlate of this behavioral difference, we counted the number of dopamine producing cells (TH-ir) in WT and PRKO mice in the SNc and VTA of sexually naive male mice. In addition, we investigated the relationship between the number of TH-ir cells and recent sexual behavioral experience by counting the number of TH-ir positive cells in males that had received four behavior tests with a sexually receptive female.

2. Materials and methods

2.1. Animals

All animals were sexually naive WT and PRKO male mice obtained from the colony of Drs. Bert O'Malley and John Lydon at Baylor College of Medicine. Males were weaned at 3 weeks of age and housed with brothers in single-sex cages of two to four animals. Generation of PR deficient mice has previously been described [27]. Animals used in this study were approximately F10 of a 129SvEv × C57BL6 background from an initial cross of an F0 male chimera, generated by gene targeting, and a C57BL6 female. This initial cross generated 50% 129SvEv and 50% C57BL6 F1 heterozygotes that were subsequently crossed to generate F2. Generations subsequent to the F2 resulted from matings of either cousins or siblings.

Animals arrived at the University of Texas when they were 50–90 days old and were housed individually in separate polyvinyl cages on a 12-h light:12-h dark cycle with food and water provided ad libitum. Males were acclimated to the housing conditions for 3 weeks prior to the commencement of behavioral testing. Experimental protocols adhered to institutional guidelines and NIH Guidelines for the Use of Animals in Research.

2.2. Experiment 1

2.2.1. Behavior testing

All males were sexually naive prior to behavior testing. Males were divided into two groups. One group was injected intraperitoneally with the dopamine D1 antagonist SCH23390 (0.5 mg/kg in 0.2% ethanol) 15 min prior to a 90 min behavior test, while males in the second group were injected with vehicle (0.2% ethanol). Males were immediately returned to their home cage after injection and the cage was moved to a separate area of the colony room for testing.

Based on studies of sexual behavior in mice (reviewed in ref. [7]), we adapted our behavioral testing protocol from that described in ref. [35]. In the current study, behavioral testing was conducted in the male's home cage under red lights during the first third of the dark portion of the light:dark cycle. Males were presented with a sexually receptive, peripubertal (25–27 days old), CD-1 female mouse (Charles River Labs). Female receptivity was induced with an injection of 3 IU of pregnant mares serum gonadotropin (Sigma Chemicals) administered 48 h prior to testing and a second injection of 1 IU of human chorionic gonadotropin (HCG, Sigma Chemicals) administered 8–14 h prior to testing. On the day of testing, females were screened for receptivity with sexually experienced CF-1 males. Only receptive females, those that were mounted and intromitted by at least two different stimulus males, were used as stimulus females.

Tests lasted either 90 min or until the male ejaculated, and for each test we recorded the time to the first and total number of mounts, intromissions, and ejaculations. If a male did not mount, intromit, or ejaculate he did not receive a latency score and was given a frequency score of zero for that behavior. We define the intromission latency as the time from the first mount to the first intromission, and ejaculation latency as the time from the first intromission to ejaculation.

2.2.2. Statistics

We performed Likelihood Ratio Chi-square tests within each genotype with treatment (SCH or vehicle) as the independent variable and whether males mounted, intromitted, and ejaculated as dependent variables. In addition, among individuals that mounted on the behavior test, we performed ANOVAs with genotype and treatment as the independent variables and the number of mounts and the latency to mount as dependent variables. Because few males treated with the dopamine antagonist intromitted or ejaculated, we did not have sufficient power to investigate treatment effects on intromission or ejaculation latencies or frequencies. For all tests, we set our alpha to $P < 0.05$.

2.3. Experiment 2

2.3.1. Behavior testing

Males were divided into two groups. One group (experienced; $n = 12$/genotype) included the vehicle-treated males from the study with SCH23390. These males received a total of four behavior tests (including the first test with the vehicle injection) with a sexually receptive female each separated by 1 week. The second group (naive; $n = 8$/genotype) remained sexually naive during the 4-week test period. These males were injected with vehicle during the first week of testing to ensure similarity in handling experience. Animals from both groups were sacrificed at the same time, 1–3 h after the fourth behavior test for the experienced group.

Naive males were housed in the same room as the experienced males, were handled in a manner similar to experienced males, and were sacrificed at the same time as experienced males. However, naive males did not have the opportunity to interact with females between weaning and sacrifice.

2.3.2. Tyrosine hydroxylase (TH) immunocytochemistry

At sacrifice, males from all groups were given a lethal dose of sodium pentobarbital (0.65 g/kg) and transcardially perfused with 0.9% heparinized saline (50 ml at 8 ml/min) followed by 4% paraformaldehyde in 0.1 M PBS, pH 7.4 (250 ml at 8 ml/min). Brains were removed and soaked overnight in 4% paraformaldehyde then soaked for 24 h in 20% sucrose. Thereafter, they were frozen in isopentane and stored at −80 °C. Serial 50 μm sections were cut on a cryostat and four sets of tissue were collected and stored in antifreeze at −20 °C. Immunocytochemistry was performed on one set of free-floating 50 μm sections, and therefore sections were 200 μm apart. Sections were rinsed overnight in 0.05 M TBS (pH 7.7), then incubated in 3% hydrogen peroxide and 4% normal goat serum in TBS for 20 min. After blocking for 1 h in 4% normal goat serum with avidin, sections were incubated for 72 h at 4 °C in primary antibody (1:1000; rat anti-TH, Chemicon International, Temecula, CA) with 4% goat serum, biotin, and TBS. Sections were then incubated at room temperature in goat anti-rat secondary antibody (1:500; Vector Labs, Burlingame, CA) for 2 h followed by an incubation in avidin–biotin complex (Vector Labs ABC kit) for 2 h. Immunoreactivity was visualized using 3,3-diaminobenzedine (DAB, Vector Labs). Sections were then mounted and dehydrated onto slides and counterstained with cresyl violet. Sections incubated in 4% goat serum in the absence of primary antibody were used as negative controls; negative controls were always devoid of staining.

2.3.3. Cell counting and analysis

Slides were randomized and coded so that we were blind to the experimental groups (genotype and experience). Sections were imaged using a Zeiss microscope fitted with a Ludl Electronic Products MAC 2002 motorized stage (LEP,

NY), an Optronics DEI 750 camera (Optronics, CA), and a Dell Pentium III XPS B733r computer.

We counted the number of TH-immunoreactive cells in two nuclei in the midbrain, the VTA and the SNc. Differences in the number of TH-ir cells may be due either to differences in the expression of the enzyme or to actual differences in the number of cells capable of producing TH. Nuclei were delineated based on both TH and Nissl staining using [33]. For the SNc, we counted cells that were in the rostral most portion of the SNc that extended out from dorsolateral to ventromedial and in the VTA we counted cells that were located medially and often were located on the same sections as those with the SNc. For each individual, we counted cells unilaterally at 40× in each nucleus on all sections where the nucleus was present using StereoInvestigator software (MicroBrightfield, VT). Cells were counted manually and were considered labeled if they had dark DAB staining around the edge of the soma and dendrites and more diffuse staining in the cytoplasm. To ensure a similar threshold for considering cells as labeled, we compared examples of labeled cells representing the minimum staining intensity across sections and across individuals.

2.3.4. Statistics

2.3.4.1. Behavior. Behavior for the four behavior tests was analyzed using an ANOVA with genotype (WT or PRKO) and test (1–4) as the independent variables. Male identity was also included as a random variable nested within genotype; adding this factor eliminates the variability among subjects due to individual differences from the error term [48,49]. The dependent variables were the latency to and number of mounts and intromissions and the ejaculation latency. When the interaction between genotype and test day was significant we performed planned contrasts using Student's *t*-tests comparing the genotypes on each test day. When there was a significant effect of test day, we performed planned contrasts using Student's *t*-tests comparing tests 2–4 to test 1. For all planned contrasts, we set our α to 0.0125 (Bonferroni correction 0.05/4) to account for the increased number of comparisons. Males were only allowed to ejaculate once per test, making the ejaculation frequency an ordinal variable. Consequently, we analyzed the proportion of males that ejaculated on each test day using Likelihood Ratio Chi-square tests.

Because we also wanted to know whether there were any differences in behavior immediately prior to sacrifice that might be associated with differences in the number of TH-ir cells, we also performed ANOVAs with the latency to and number of mounts, intromissions, and ejaculations on the fourth test day as the dependent variables and genotype as the independent variable.

2.3.4.2. Number of tyrosine hydroxylase-immunoreactive cells. We performed ANOVAs with genotype and experience (naive versus experienced) as the independent variables and the number of cells in the SNc and VTA as dependent variables. When the interaction between genotype and experience was significant we performed planned contrasts, comparing the genotypes within each experience group (e.g. naive WT versus naive PRKO) and comparing each experience group within each genotype (e.g. naive WT versus experienced WT).

Finally, to investigate the relationship between neural and behavioral phenotype we performed linear regressions of the number of TH-ir cells in the SNc and VTA and the log of the latency to and number of mounts, intromissions, and ejaculations on the fourth behavior test.

3. Results

3.1. Experiment 1

Among WT males, significantly more males treated with vehicle intromitted relative to males treated with the D1 antagonist (Fig. 1A; $\chi^2 = 4.27$, $P = 0.039$). In addition, there was a trend toward fewer SCH treated WT males ejaculating relative to vehicle-treated males ($\chi^2 = 3.02$, $P = 0.082$). Drug treatment had no effect on the percent of PRKO males mounting, intromitting or ejaculating. The D1 antagonist also resulted in fewer

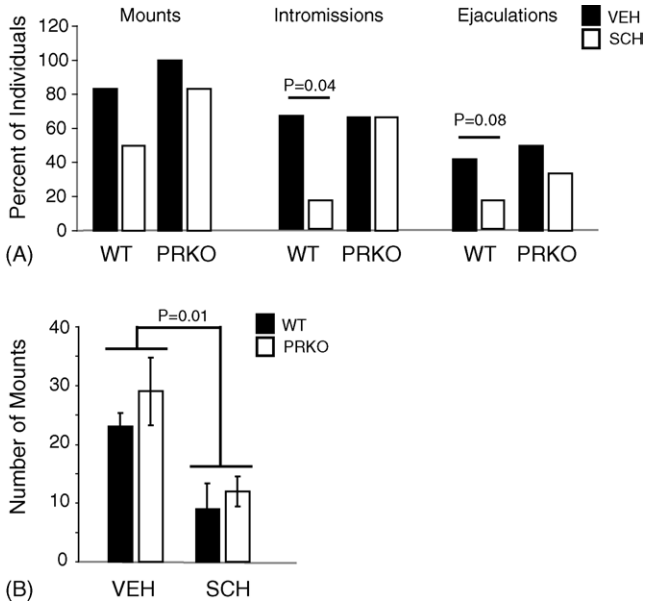

Fig. 1. Sexual behavior of WT and PRKO mice given either the dopamine D1 antagonist SCH 23390 or vehicle. (A) The percent of individuals of each genotype showing mounts, intromissions, and ejaculations after treatment with vehicle (black bars) or SCH 23390 (white bars). (B) Number of mounts to ejaculation for WT (black bars) and PRKO mice (white bars) given vehicle or SCH 23390 prior to testing.

mounts in both genotypes (Fig. 1B; $F = 6.9$, $P = 0.014$) but had no effect on the latency to mount.

3.2. Experiment 2

3.2.1. Behavior

There was a significant effect of genotype on the ejaculation latency (Fig. 2A; $F = 10.65$, $P = 0.002$), with PRKO males taking longer to ejaculate than WT males. There was a significant effect of test day on the intromission latency (Fig. 2B; $F = 6.02$, $P = 0.002$), with shorter latencies on test 2 ($t = 3.40$, $P = 0.001$), test 3 ($t = 3.88$, $P = 0.003$), and test 4 ($t = 3.31$, $P = 0.002$) than on test 1 for both genotypes.

There was a trend toward a significant genotype by test day interaction for the number of mounts ($F = 2.45$, $P = 0.07$) and planned contrasts revealed that PRKO mice mounted more often, though not significantly with the Bonferroni correction, than WT mice on test 4 (Fig. 2C; $t = 2.25$, $P = 0.028$). Similarly, there was also a trend toward more intromissions by PRKO than WT males (Fig. 2C; $t = 3.53$, $P = 0.067$).

We found that on the fourth test, PRKO mice took longer to ejaculate than WT mice (Fig. 3A; $F = 7.09$, $P = 0.022$). In addition, PRKO mice mounted (Fig. 3B; $F = 7.95$, $P = 0.01$) and intromitted (Fig. 3B; $F = 6.03$, $P = 0.023$) significantly more frequently than WT mice.

3.2.2. Number of tyrosine hydroxylase-immunoreactive cells

In the SNc, there was a significant interaction between genotype and experience ($F = 6.93$, $P = 0.01$). Experienced PRKO males had more TH-ir cells in the SNc than naive PRKO males

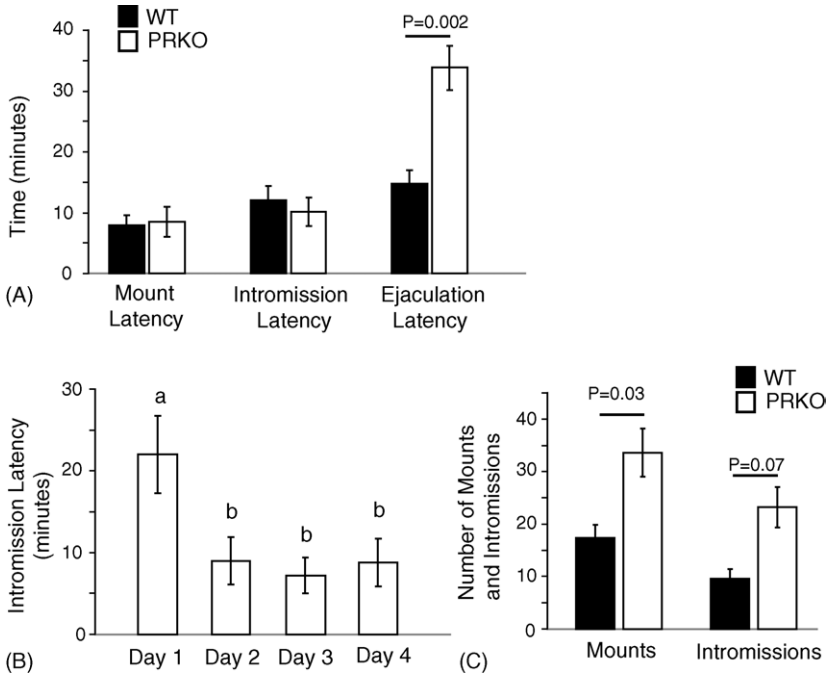

Fig. 2. Sexual behavior of WT and PRKO mice across all four behavior tests. (A) Across all four tests, PRKO males (white bars) had significantly higher ejaculation latencies relative to WT males (black bars), while there were no differences in the time to the first mount, or the time from first mount to first intromission. (B) The only behavioral parameter to show a significant effect of sexual experience was the intromission latency. Data for both genotypes is pooled. Different letters indicate groups that are significantly different ($P < 0.01$). Both WT and PRKO males showed decreases in intromission latency on tests 2–4 relative to test 1. (C) Across all four tests, PRKO males (white bars) engaged in more mounts than did WT males (black bars). A similar, though not significant trend, was also apparent in the number of intromissions.

(Fig. 4A; $t = 4.79$, $P = 0.04$), while there were no differences among WT males. In addition, there was a trend toward more cells in experienced PRKO males relative to experienced WT males (Fig. 4A; $t = 3.29$, $P = 0.06$). In the VTA there was a significant interaction between genotype and experience ($F = 4.01$, $P = 0.05$). Planned contrasts revealed that experienced PRKO males had significantly more cells than experienced WT males (Fig. 4B; $t = 6.43$, $P = 0.02$).

There was a significant correlation between the log of the latency to mount on the final behavior test and the number of cells in the SNc in PRKO but not WT males (Fig. 4C; $R^2 = 0.84$, $F = 31.62$, $P = 0.001$). There were no significant correlations between any of the behavioral measures and the number of cells in the VTA for either WT or PRKO males.

4. Discussion

While there is considerable information on the role of PR in the display of sexual behavior in females [28], the role of PR in modulating sexual behavior in males has only begun to be elucidated. Here we report that PRKO males require more mounts and intromissions and have longer latencies prior to ejaculation than do WT males. This effect was present across all four behavior tests, and was statistically significant on the fourth behavior test. However, it is unclear whether this behavioral difference is a result of the absence of PR during adulthood or development. Studies in rats have demonstrated that adult males have low mount frequencies when given the PR antagonist RU486 [51]. However, it is also the case that blocking PR with RU486 perina-

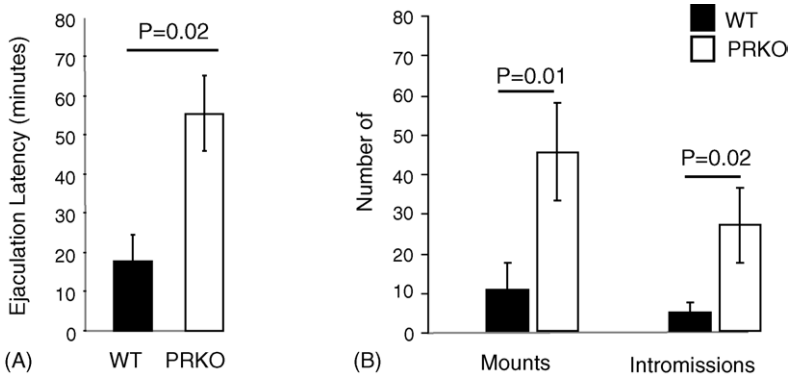

Fig. 3. Sexual behavior of WT and PRKO males on the final behavior test. (A) PRKO males took significantly longer to ejaculate than WT males. (B) PRKO males (white bars) mounted and intromitted females significantly more often than WT males (black bars).

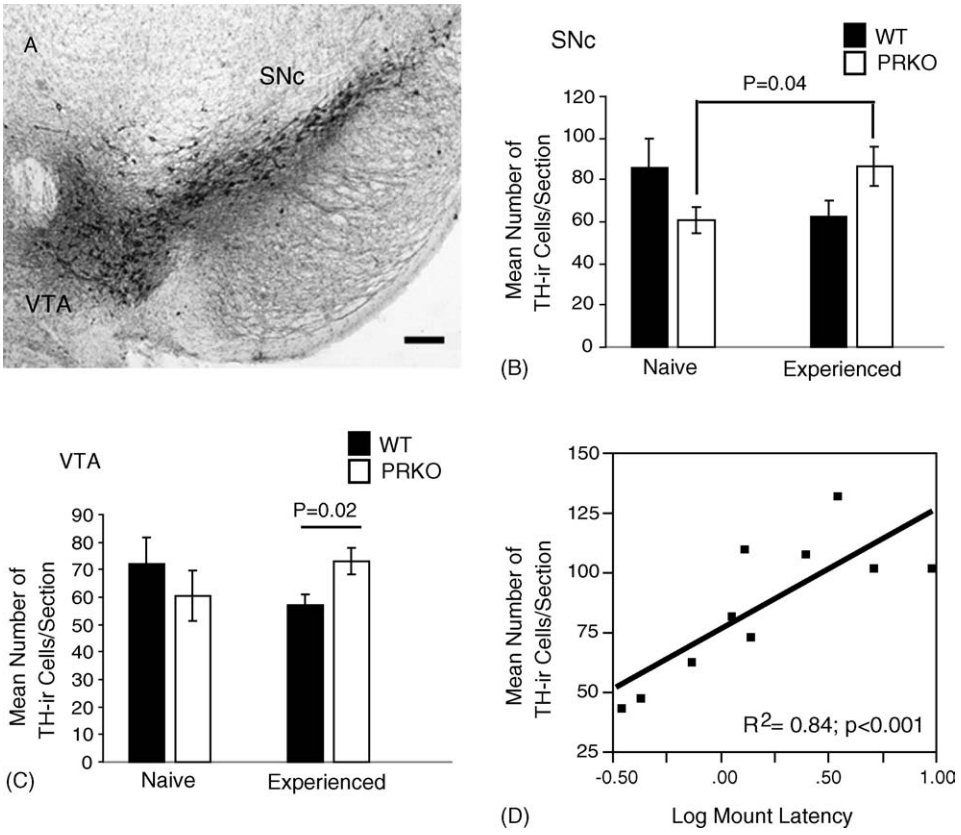

Fig. 4. Number of TH-ir cells in the SNc and VTA of naive and experienced WT and PRKO males. (A) Photomicrograph of TH staining in the SNc and VTA. Scale bar = 100 μm. (B) In the SNc, experienced PRKO males had significantly more TH-ir cells than naive PRKO males, and there was a non-significant trend ($P = 0.06$) toward more cells in experienced PRKO males than in experienced WT males. (C) In the VTA, experienced PRKO males had significantly more TH-ir cells than experienced WT males. (D) Among PRKO mice there was a significant, positive correlation between the latency to mount and the number of TH-ir cells in the SNc.

tally results in fewer males showing ejaculatory behavior when tested as adults [26]. Our data differ from both of these examples as PRKO males copulate, but take more time and require more mounts and intromissions to do so. Manipulations of PR expression during different developmental epochs may help to elucidate the role of PR in modulating the display of male sexual behavior.

We also found genotype differences in the expression of the dopamine synthesizing enzyme tyrosine hydroxylase in the VTA and SNc. In the VTA, the number of TH-ir cells was significantly higher in experienced PRKO males than in experienced WT males, while in the SNc, experienced PRKO males had more TH-ir cells than naive PRKO males and there was a trend towards more TH-ir cells in experienced PRKO males relative to experienced WT males. Given that the main differences between the genotypes occur among males that have received social and sexual experience, it is possible that the differences in TH expression arise as a consequence of behavioral experience. Across all 4 weeks of testing, PRKO males had longer latencies to ejaculate and on the final day of testing PRKO males displayed significantly more mounts and intromissions than WT mice. We also found that the number of TH-ir cells was positively correlated with the latency to mount and intromit in PRKO but not WT mice. Sexual experience has been shown to alter neural phenotype in a number of species. Sexually experienced males are more resilient to lesions of neural areas involved in sexual behavior [1,12,18,22,29] and have smaller cell somata and nuclei in the spinal nucleus of the bulbocavernosus [6]. In addition, metabolic activity in the preoptic area of sexually experienced lizards and rats is higher than in sexually naive males [43,44]. It is possible that the genotype differences in behavior or differences in TH-ir cell plasticity due to the absence of PR may drive differences in TH-ir expression among experienced males. Such an effect would be of particular interest because, while there is considerable work demonstrating that decreases in the number of TH-ir cells, such as those that occur in the SNc with Parkinson's disease, MPTP treatment, or 6-hydroxydopamine lesions of the striatum, can affect movement and behavior [14,21,47], there is less support for the idea that differences in behavioral performance may affect the number of TH-ir cells.

The genotype differences in neural phenotype may also be a consequence genotype differences in plasticity of TH-ir cells resulting from the absence of the PR during development. In mice, PR is expressed perinatally in the midbrain [5] and PR expression peaks at a time when the number and activity of midbrain TH neurons are increasing to adult levels and beginning to form functional contacts in the striatum [2]. Thus, genotype differences in TH-ir cell number may result as a consequence of the absence of PR during this developmental epoch. Estrogen receptor (ER) expression also increases during this time

[38], and ER is regulated by, and often requires, the cooperative action of PR [15]. Estrogen stimulation of midbrain neurons during development affects the differentiation of dopaminergic cells and increases TH mRNA expression [39], alters dopamine uptake [13], and regulates neurite outgrowth of TH-ir neurons [40]. Thus, in the absence of regulation by PR, ER may differentially affect TH expression or cell morphology. It will be interesting to investigate whether acute manipulation of PR during development and in adulthood also affects neural changes in response to social and sexual experience and in addition whether other aspects of these nuclei differ between the genotypes, for example the number of GABAergic interneurons or nuclear volume.

Among PRKO males there was a positive correlation between the number of TH-ir cells in the SNc and the latency to mount, indicating that males with potentially higher levels of dopamine release into the striatum took longer and required more time interacting with the female to initiate copulation. In addition, across all four tests, PRKO males generally required more mounts and intromissions and a longer latency to ejaculate than did WT males. The greater sensory feedback and stimulation necessary for copulation in PRKO males may be an indication of a genotype difference in sensory processing or sensorimotor integration. One possibility is that the genotypes differ in the regulation or expression of dopamine receptors in areas involved in motivation and sensorimotor integration including the nucleus accumbens and striatum. Cells in the striatum respond to somatosensory stimuli and other sensory inputs [50] and these responses are altered by stimulation of the VTA and SNc. In addition, midbrain dopaminergic cells in the SNc and VTA are responsive to a variety of types of stimuli. Thus, lower levels of dopamine D1 receptor in the striatum or nucleus accumbens in PRKO males could affect processing of sensory information and could underlie their need for greater sensory input prior to ejaculation. Such a difference in receptor expression could also explain the lower sensitivity of PRKO males to the D1 antagonist, as evidenced by the antagonist decreasing the percent of WT males, but not PRKO males, displaying intromissions and ejaculations. While it is not known whether there are genotype differences in dopamine receptor expression, one possibility is that the small differences in the number of dopamine producing cells in the SNc may be associated with differences in receptor expression in the striatum, and thus the genotype difference in behavior and sensitivity to the DA antagonist.

In both genotypes, treatment with the D1 antagonist decreased the number of mounts but did not affect the latency to mount. Mount latency and frequency may be indicators of sexual motivation, but are also affected by an individual's general arousal and locomotor ability. We did not test the effect of the antagonist on other behaviors, and it is possible that the decreases in mount frequency in both genotypes as well as the failure of many WT males to mount and intromit is not specifically a deficit in sexual behavior but may reflect more general effects of the antagonist on sexual motivation or locomotion. Moreover, given the differences in TH expression in the SNc and VTA, which are areas associated with both motivational and motor aspects of behavior, it will be interesting to determine whether there are genotype differences in other behaviors that recruit these neural systems.

Previous work in this laboratory [35] found that on their first behavioral test PRKO mice had lower mount frequencies than WT mice, while on subsequent behavioral tests there was no difference in mounting between WT and PRKO males. In contrast, we found no significant differences in mounting between the genotypes on the first day, though there were trends toward more mounts and intromissions and longer ejaculation latencies by PRKO males, and by the fourth test, these trends were statistically significant. There are a number of procedural differences in the handling and testing of the males between the two studies that may account for the differences in results. For example, males in the current study were tested under red light illumination during the dark portion of the light:dark cycle, while those in ref. [35] were tested under fluorescent lights during the first 3 h after lights came on. Time of day and illumination have been shown to affect behavior in ERβKO mice [23,30–32]. In addition, in order to increase the probability that males would ejaculate on the first test, our tests lasted 90 min rather than 20 min. Finally, whereas males in our study received four behavior tests each separated by 1 week, males in ref. [35] were tested every day for 4 days. These changes in testing condition were implemented in an effort to make this study more comparable to other studies of sexual behavior in mice [7]. However, the behavior of knockout mice has been shown to be quite sensitive to variation in testing conditions [49] and these dramatic differences in testing may explain why our data do not replicate those of ref. [35].

Based on the current study we cannot discern the time course or amount of behavioral experience necessary for the changes in TH. Among sexually experienced rats, changes in the expression of the immediate early gene FOS can occur after a single ejaculation [4,10]. Similarly, in male Japanese quail, sexual behavior induces expression of both FOS and EGR-1 in catecholaminergic cells in areas such as the VTA [9]. The TH gene has an AP-1 site which binds c-fos and TH transcription can be activated by both egr-1 and c-fos in vitro [16,17,34]. Thus, it is possible that through the effects transcription factors such as c-fos or egr-1 or through other rapidly changing second-messenger systems such as cyclic AMP, changes in TH expression could occur after only minimal sexual experience. Additional manipulations of sexual experience, for example providing males with fewer behavior tests, would provide insight into how rapidly changes in TH expression occur, while increasing the time from the final sexual experience to sacrifice would indicate how persistent the changes in TH are. Finally, because all males ejaculated on at least one test, we cannot determine which aspects of interacting with a female are paramount to producing the changes in TH between naive and experienced males, in particular whether sexual experience, social experience, or simply an increase in motoric activity is necessary for the changes in TH expression.

Acknowledgements

NIMH T32 18837 and F31 12716 (SCW), HD 07857 and HD 007495 (BOM) and MH 41770 (DC). We would like to thank Jon T. Sakata for his critique of earlier versions of the manuscript.

References

[1] Arendash GW, Gorski RA. Effects of discrete lesions of the sexually dimorphic nucleus of the preoptic area or other medial preoptic regions on the sexual behavior of male rats. Brain Res Bull 1983;10:147–54.

[2] Baker H, Joh TH, Reis DJ. Time of appearance during development of differences in nigrostriatal tyrosine hydroxylase activity in two inbred mouse strains. Dev Brain Res 1982;4:157–65.

[3] Balthazart J, Castagna C, Ball GF. Differential effects of D1 and D2 receptor agonists and antagonists on appetitive and consummatory aspects of male sexual behavior in Japanese quail. Physiol Behav 1997;62:571–80.

[4] Baum MJ, Everitt BJ. Increased expression of c-fos in the medial preoptic area after mating in male rats: role of afferent inputs from the medial amygdala and midbrain tegmental field. Neuroscience 1992;50:627–46.

[5] Beyer C, Damm N, Brito V, Kuppers E. Developmental expression of progesterone receptor isoforms in the mouse midbrain. Neuroreport 2002;13:877–80.

[6] Breedlove SM. Sex on the brain. Nature 1997;389:801.

[7] Burns-Cusato M, Scordalakes EM, Rissman EF. Of mice and missing data: what we know (and need to learn) about male sexual behavior. Physiol Behav 2004;83:217–32.

[8] Castagna C, Ball GF, Balthazart J. Effects of dopamine agonists on appetitive and consummatory male sexual behavior in Japanese quail. Pharmacol Biochem Behav 1997;58:403–14.

[9] Charlier TD, Ball GF, Balthazart J. Sexual behavior activates the expression of the immediate early genes c-fos and Zenk (egr-1) in catecholaminergic neurons of male Japanese quail. Neuroscience 2005;131: 13–30.

[10] Coolen LM, Peters HF, Veening JG. Fos immunoreactivity in the rat brain following consummately elements of sexual behavior: a sex comparison. Brain Res 1996;738:67–82.

[11] Damsma G, Pfaus JG, Wenkstern D, Phillips AG, Fibiger HC. Sexual behavior increases dopamine transmission in the nucleus accumbens and striatum of male rats: comparison with novelty and locomotion. Behav Neurosci 1992;106:181–91.

[12] de Jonge FH, Louwerse AL, Ooms MP, Evers P, Endter E, van der Poll N. Lesions of the SDN-POA inhibit sexual behavior of male wistar rats. Brain Res Bull 1989;23:483–92.

[13] Engele J, Pilgrim C, Reisert L. Sexual differentiation of mesencephalic neurons in vitro: effects of sex and gonadal hormones. Int J Neurosci 1989;7:603–11.

[14] Erwan B, Dovero S, Prunier C, Ravenscroft P, Chalon S, Guilloteau D, et al. Relationship between the appearance of symptoms and the level of nigrostriatal degeneration in a progressive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine lesioned Macaque model of Parkinson's disease. J Neurosci 2001;21:6583–861.

[15] Fitzpatrick SL, Berrodin TJ, Jenkins SF, Sindoni DM, Deecher DC, Frail DE. Effect of estrogen agonists and antagonists on induction of progesterone receptor in a rat hypothalamic cell line. Endocrinology 1999;140:3928–37.

[16] Ghee M, Baker H, Miller JC, Ziff EB. AP-1, CREB and CBP transcription factors differentially regulate the tyrosine hydroxylase gene. Brain Res Mol Brain Res 1998;30:101–14.

[17] Gizang-Ginsberg E, Ziff EB. Fos family members successively occupy the tyrosine hydroxylase gene AP-1 site after nerve growth factor or epidermal growth factor stimulation and can repress transcription. Mol Endocrinol 1994;8:249–62.

[18] Harris VS, Sachs BD. Copulatory behavior in male rats following amygdaloid lesions. Brain Res 1975;86:514–8.

[19] Hull EM, Du J, Lorrain DS, Matuszewich L. Extracellular dopamine in the medial preoptic area: implications for sexual motivation and hormonal control of copulation. J Neurosci 1995;15:7465–71.

[20] Hull EM, Muschamp JW, Sato S. Dopamine and serotonin: influences on male sexual behavior. Physiol Behav 2004;83:291–307.

[21] Jenner PDS. The MPTP-treated primate as a model of motor complications in PD: primate model of motor complications. Neurology 2003;61(S3):S4–11.

[22] Kondo Y. Lesions of the medial amygdala produce severe impairment of copulatory behavior in sexually inexperienced male rats. Physiol Behav 1992;51:939–43.

[23] Krezel W, Dupone S, Krust A, Chambon P, Chapman PF. Increased anxiety and synaptic plasticity in estrogen β-deficient mice. Proc Natl Acad Sci USA 2001;98:12278–82.

[24] Lindzey J, Crews D. Hormonal control of courtship and copulatory behavior in male *Cnemidophorus inornatus*, a direct ancestor of a unisexual, parthenogenic lizard. Gen Comp Endocrinol 1986;64:411–8.

[25] Lindzey J, Crews D. Effects of progesterone and dihydrotestosterone on stimulation of androgen-dependent sex behavior, accessory sex structures, and in vitro binding characteristics of cytosolic androgen receptors in male whiptail lizards (*Cnemidophorus inornatus*). Horm Behav 1993;27:269–81.

[26] Lonstein J, Quadros PS, Wagner CK. Effects of neonatal RU486 on adult sexual, parental, and fearful behaviors in rats. Behav Neurosci 2001;115:58–70.

[27] Lydon J, DeMayo F, Funk C, Mani S, Hughes A, Montgomery C, et al. Mice lacking progesterone receptor exhibit pleitropic reproductive abnormalities. Genes Dev 1995;9:2255–78.

[28] Mani S. Emerging concepts in the regulation of female sexual behavior. Scand J Psychol 2003;44:231–9.

[29] Merideth M. Vomeronasal organ removal before sexual experience impairs male hamster mating behavior. Physiol Behav 1986;36:737–43.

[30] Nomura M, Durbak L, Chan J, Smithies O, Gustafsson JA, Korach KS, et al. Genotype/age interactions on aggressive behavior in gonadally intact estrogen receptor β knockout (βERKO) male mice. Horm Behav 2002;41:288–96.

[31] Ogawa S, Chan J, Chester AE, Gustafsson JA, Korach KS, Pfaff DW. Survival of reproductive behaviors in estrogen β gene-deficient (βERKO) male and female mice. Proc Natl Acad Sci USA 1999;96: 12887–92.

[32] Ogawa S, Chan J, Gustafsson JA, Korach KS, Pfaff DW. Estrogen increases locomotor activity in mice through estrogen receptor α: specificity for the type of activity. Endocrinology 2003;144:230–9.

[33] Paxinos G, Franklin KBJ. The mouse atlas in stereotaxic coordinates. 2nd ed. Academic Press; 2000.

[34] Papanikolaou NA, Sabban EL. Ability of Egr1 to activate tyrosine hydroxylase transcription of PC12 cells. Cross-talk with AP-1 factors. J Biol Chem 2000;275:26683–9.

[35] Phelps S, Lydon J, O'Malley BW, Crews D. Regulation of male sexual behavior by progesterone receptor, sexual experience, and androgen. Horm Behav 1998;34:294–302.

[36] Quadros PS, Goldstein AY, De Vries GJ, Wagner CK. Regulation of sex differences in progesterone receptor expression in the medial preoptic nucleus of postnatal rats. J Neuroendocrinol 2002;14:761–7.

[37] Quadros PS, Pfau JL, Goldstein AY, De Vries GJ, Wagner CK. Sex differences in progesterone receptor expression: a potential mechanism for estradiol-mediated sexual differentiation. Endocrinology 2002;143:3727–39.

[38] Raab H, Karolczak M, Reisert I, Beyer C. Ontogenetic expression and splicing of estrogen receptor-alpha and beta mRNA in the rat midbrain. Neurosci Lett 1999;275:21–4.

[39] Raab H, Pilgrim C, Reisert I. Effects of sex and estrogen on tyrosine hydroxylase mRNA in cultured embryonic rat mesencephalon. Mol Brain Res 1995;33:157–64.

[40] Reisert I, Han V, Lieth E, Toran-allerand CD, Pilgrim C, Lauder JM. Sex steroids promote neurite outgrowth in mesencephalic tyrosine hydroxylase immunoreactive neurons in vitro. Int J Dev Neurosci 1987;5:91–8.

[41] Sakamoto H, Ukena K, Tsutsui K. Effects of progesterone synthesized de novo in the developing Purkinje cell on its dendritic growth and synaptogenesis. J Neurosci 2001;21:6221–32.

[42] Sakamoto H, Ukena K, Tsutsui K. Dendritic spine formation in response to progesterone synthesized de novo in the developing Purkinje cell in rats. Neurosci Lett 2002;322:111–5.

[43] Sakata JT, Gonzalez-Lima F, Gupta A, Crews D. Repeated interactions with females elevate metabolic capacity in the limbic system of male rats. Brain Res 2002;936:27–37.

[44] Sakata JT, Gupta A, Gonzalez-Lima F, Crews D. Heterosexual housing increases the retention of courtship behavior following castration and elevates metabolic capacity in limbic brain nuclei in male whiptail lizards, *Cnemidophorus inornatus*. Horm Behav 2002;42:263–73.
[45] Schultz W. Dopamine neurons and their role in reward mechanisms. Curr Opin Neurobiol 1997;7:191–7.
[46] Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. Science 1997;275:1593–9.
[47] Schwarting JKW, Huston JP. The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatment. Prog Neurobiol 1996;50:275–331.
[48] Sokal RR. Rohlf. Biometry. New York: W.H. Freeman; 1995.
[49] Stevens J. Applied multivariate statistics for the social sciences. 3rd ed. New Jersey: Lawrence Erlbaum Associate, Inc.; 1996.
[50] Wahlsten D. Standardizing tests of mouse behavior: reasons, recommendations, and reality. Physiol Behav 2001;73:695–704.
[51] West CHK, Micheal RP. Responses of units in the mesolimbic system to olfactory and somatosensory stimuli: modulation of sensory input by ventral tegmental stimulation. Brain Res 1990;532:307–16.
[52] Witt DM, Young LJ, Crews D. Progesterone modulation of androgen-dependent sexual behavior in male rats. Physiol Behav 1995;57:307–13.
[53] Woolley SC, Crews D. Species differences in the regulation of tyrosine hydroxylase in *Cnemidophorus* whiptail lizards. J Neurobiol 2004;60:360–8.
[54] Woolley SC, Sakata JT, Crews D. Tyrosine hydroxylase expression is affected by sexual vigor and social environment in male *Cnemidophorus inornatus*. J Comp Neurol 2004;476:429–39.
[55] Woolley SC, Sakata JT, Crews D. Evolutionary insights into the regulation of courtship behavior in male amphibians and reptiles. Physiol Behav 2004;83:347–60.
[56] Woolley SC, Sakata JT, Gupta A, Crews D. Evolutionary changes in dopaminergic modulation of courtship behavior in *Cnemidophorus* whiptail lizards. Horm Behav 2001;40:483–9.
[57] Young LJ, Greenberg N, Crews D. The effects of progesterone on sexual behavior in male green anole lizards (*Anolis carolinensis*). Horm Behav 1991;25:477–88.