Hormones and Behavior **34,** 294–302 (1998)
Article No. HB981485

Regulation of Male Sexual Behavior by Progesterone Receptor, Sexual Experience, and Androgen

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Received March 9, 1998; revised June 18, 1998; accepted September 15, 1998

Recent studies have demonstrated that physiological doses of progesterone may facilitate the androgen-dependent display of male sexual behavior in laboratory rats and three species of lizard. We used mice with a targeted disruption of the progesterone receptor to investigate whether such interactions exist in male mice and whether they may be modified by sexual experience. We found that naive intact male progesterone receptor knockout (PRKO) mice exhibit reduced mount frequencies compared to wild-type (WT) mice. Also unlike WT mice, sexually experienced PRKO males show profound losses in many measures of sexual behavior following castration. In a second experiment, we tested whether male mice heterozygous for a null mutation at the progesterone receptor locus were responsive to testosterone and progesterone treatment. We found that heterozygous males showed a reduced response to testosterone. The data are consistent with experiments indicating that the progesterone receptor is able to facilitate male-typical sex behaviors in other species and suggest novel mechanisms underlying the interaction of androgens and experience.

Key Words: **progesterone receptor; male; sexual behavior; castration; knockout; mouse.**

The sources and mechanisms of individual variation represent a major challenge to the behavioral sciences. Within the study of masculine sexual behavior, there are two related examples of individual differences that are as salient as they are poorly understood: variation in the capacity of experienced males to exhibit sexual behaviors following castration, and variation in the sensitivity of males to androgen treatment (Sachs and Meisel, 1994). It is well documented that testosterone (T) may elicit male-typical behaviors by binding androgen receptor (AR) or by being aromatized and binding one of the estrogen receptor subtypes (ER) (Baum, Tobet, Starr, and Bradshaw, 1982; Christensen and Clemens, 1974). Although receptor abundance relates to the performance of male behaviors (Clark, Davis, and Roy, 1985), there is a great deal of variability that does not seem explicable simply in terms of circulating androgens (Beach and Holz-Tucker, 1949; Beach and Fowler, 1959; Damassa, Smith, Tennent, and Davidson, 1977) or receptor expression (Clemens, Wee, Weaver, Roy, Goldman, and Rakerd, 1988). What mechanisms are responsible for intraspecific variation in the display of male sexual behaviors, and how do they interact with androgen-dependent processes?

One obvious line of inquiry is to determine how variation in past experience might shape sexual behaviors. As in many vertebrate species, sexually experienced male mice exhibit sexual behavior following castration at much higher levels than do sexually naive males (Sachs and Meisel, 1994). Classic work in behavioral genetics has shown heritable strain differences in the influence experience exerts over androgen dependence (reviewed in McGill, 1965).

We sought to investigate a novel potential source for individual differences—the progesterone receptor (PR)—using male mice with a null mutation at the PR locus. This model system allowed us to investigate the contributions PR makes to the sex behavior of males with varied experience and plasma steroids.

Named for its central role in female reproduction,

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0018-506X/98 $25.00

progesterone (P) traditionally has been thought to have little or no function in the control of sexual behavior in males. Indeed, early experiments indicated that administration of P to male birds and rats will inhibit their sexual behavior (Erickson, Bruder, Komisaruk, and Lehrman, 1967; Erpino, 1967, 1973; Bottoni, Lucini, and Massa, 1985). This viewpoint has become so accepted that it serves as a rationale for the use of progestins in the "chemical castration" of sex-offenders (Bradford, 1988; Lehne, 1988). However, the physiology of P secretion reveals a marked diurnal rhythm in P secretion in male rats (Kalra and Kalra, 1977) and humans (Vermeulen and Verdonck, 1976; Kage, Fonner, Weber, and Schoneshofer, 1982; Opstad, 1994). In addition, work with several species of reptiles has demonstrated that exogenous P, whether administered systemically or directly into the brain, will stimulate courtship and copulatory behavior in castrated males (Lindzey and Crews, 1986, 1988, 1992; Young, Greenberg, and Crews, 1991; Crews, Godwin, Hartman, Grammer, Prediger, and Sheppherd, 1996) and that T and P can synergize in stimulating sexual behavior in males (Young *et al.*, 1991; Lindzey and Crews, 1988, Lindzey and Crews, 1992) much as estrogen (E) and P synergize in stimulating sexual behavior in female rodents (Pfaff, Shwartz-Giblin, McCarthy, and Kow, 1994). These data prompted a reassessment of the evidence gleaned from mammalian work and the discovery that most data were derived from pharmacological doses of P and from the administration of synthetic progestins that have antiandrogenic properties (reviewed in Witt, Young, and Crews, 1994). Recent studies demonstrate that P administered systemically to produce physiological titers, or directly into the preoptic area (POA), stimulates the expression of sexual behavior of intact and castrated male rats (Witt, Young, and Crews, 1995; Witt, Reigada, and Wengroff, 1997); further, as in reptilian studies, T and P treatments synergize to stimulate male sexual behavior in castrated rats (Witt *et al.*, 1995).

Progesterone's role in the control of male sexual behavior has been most closely examined in reptilian models; these studies indicate that P may function by enhancing the responsiveness of the POA to androgens (Crews *et al.*, 1996). Although this has yet to be established in rodents, it is worth noting that researchers working with rats have recently found that injections into the POA of P enhance male-typical sex behavior, while injections of a P antagonist, RU 38486, impair performance (Witt *et al.*, 1997). Finally, neonatal male rats have substantially higher levels of PR in the medial POA than do female rats (Wagner, Nakayama, and De Vries, 1998). Taken together, these facts are consistent with the hypothesis that P plays a functional role in the regulation of sexual behavior in males.

We investigated the role of PR in the regulation of male sexual behavior with two studies. The first compared the sexual behavior of male mice that were homozygous for either a wild-type (WT) allele or a progesterone receptor knockout (PRKO) allele. For both genotypes, the sexual behaviors of naive and experienced intact males were compared to the behaviors of naive and experienced castrated males.

Previous studies have demonstrated that female mice heterozygous for a PR knockout allele show impaired PR expression in response to estrogen treatment, but have not been shown to exhibit deficiencies in intact behaviors (Mani, Blaustein, and O'Malley, 1997). In the second study, we sought to test whether subtle genetic deficits in PR were capable of impairing the responses of males to T replacement. To assess this possibility, we tested the sexual behaviors of male mice that were either WT or heterozygous for a PR knockout allele (HTZ). Males were tested when intact, castrated, and treated with some combination of T, P, and blank (Bl) capsules. Because intact mice heterozygous for disruptions at other loci may show normal levels of behaviors (e.g., Ogawa, LuBahn, Korach, and Pfaff, 1997), which presumably reflect a capacity to compensate for long-term deficits through regulatory mechanisms, we expected that the effects of genotype would be weak or absent from intact and castrated animals, but would be plainly manifest in response to P and T treatment.

METHODS

Generation of Knockout Mice

Generation of the progesterone receptor-deficient mouse model has previously been described (Lydon, DeMayo, Funk, Mani, Hughes, Montgomery, Shyamala, Conneely, and O'Malley, 1995). The animals used in this study were approximately F_8 of a "mixed" 129SvEv × C57BL6 background from an initial cross between an F_0 male chimera (generated by gene-targeting) and a C57BL6 female. This cross generated heterozygotes (F_1) that were 50% 129SvEv/50% C57BL6 and were subsequently crossed to generate F_2. Subsequent to F_2, either cousins or siblings were mated to generate the next generations. Since all sub-

jects were derived from the same crossings, genetic background effects should be limited to linked loci. Conditional knockouts and backcrossed strains are being developed to address this caveat.

Study 1: Experience and Castration in WT versus PRKO Males

Behavioral paradigm. Nineteen WT and 20 PRKO males were divided into a total of four treatment groups. Fourteen WT and 15 PRKO males were given sexual experience prior to castration; 5 WT and 5 PRKO males were castrated without sexual experience. Males assigned to the experience treatment were tested four times prior to castration, once per day for 4 consecutive days. Data from "naïve" males were obtained on the first day of testing; data from "experienced" males were collected from the same males and averaged over tests 2–4. All 39 males were castrated. One WT and one PRKO male, both experienced, died under anesthesia. The remaining 37 males were tested at 3 weeks following castration.

Stimulus animals were peripubertal (27– 30 days) CF-1 females supplied by Charles River Laboratories. They were injected with 3 units of gonadotropin from pregnant mare serum (PMSG, from Sigma) 48 h prior to testing, followed by 1 unit human chorionic gonadotropin (HCG, Sigma) 8–12 h prior to testing. Females were screened with sexually experienced CF-1 males to ensure receptivity. A female was judged receptive if two different stud males were able to mount and intromit the female. Receptive females were used on consecutive tests within a day unless a subject ejaculated or unless a subject was particularly aggressive, either of which would render the females unreceptive with subsequent males.

Animals were housed on a 12:12 light:dark cycle. Most subjects were tested within 2 h of light onset, all within 3 h. The order of testing was randomized from day to day to ensure that any interaction between time of testing and treatment was unbiased with respect to hormone treatment and genotype. All males were housed under testing conditions for at least 2 weeks prior to testing.

Testing was conducted in the home cage of the subject. Tests lasted 20 min or until the subject ejaculated. During the test, the mount, intromission, and ejaculation frequencies were measured. The frequency of a behavior was defined as the number of behaviors performed in a 5-min interval and was calculated by dividing the total number of observed behaviors over the test length (20 min or until ejaculation) and multiplying by 5. The latencies to first performance of each of these behaviors were also recorded. If a behavior was not performed, a latency score for that behavior was assigned a value of 1200 s—the maximal score possible in a 20-min test.

TABLE 1

Mount, Intromission, and Ejaculation Frequencies ($\mu \pm$ SE) in Wildtype (WT) and Heterozygote (HTZ) Male Mice Following Testosterone (T) Replacement; Control Animals Received Blank (BL) Implant

Genotype	Treatment	*n*	MF%	IF%	EF%
WT	T	10	223 ± 46	301 ± 105	1166 ± 560*
HTZ	T	9	171 ± 14	131 ± 25	268 ± 199*
WT	BL	9	86 ± 38	30 ± 14	18 ± 14
HTZ	BL	11	74 ± 27	31 ± 13	3 ± 3

Note. Frequencies have been averaged over tests 8 and 9 and are expressed as a percentage of behavioral frequencies measured while subjects were intact (average of tests 1–4). Numbers have been pooled with respect to progesterone treatment. Ejaculation frequencies were significantly different between WT and HTZ males treated with T ($P < 0.05$).

Statistical analysis. We compared mount, intromission, and ejaculation frequencies and latencies of naive (test 1) WT and PRKO males using *t* tests. To determine whether experience produced differences between the genotypes, we used one-tailed tests to compare these measures in the same males, but took the averages of their behaviors over tests 2–4, which were conducted while the males were intact. To determine whether WT and PRKO males differed in their responses to castration and experience, we performed a two-way ANOVA on the data from the 3-week postcastration test, with genotype and prior experience as independent variables. Where we detected genotype by experience interactions among castrates, we performed two-tailed *t* tests to compare the behaviors of experienced males.

Results. We found that naive WT males had significantly higher mount frequencies than naive PRKO males (one-tailed, $P < 0.05$; Table 1). The effect, however, was relatively subtle compared to the results of the castration manipulations. Mount frequency differences were not apparent in the averages of tests 2– 4. Sexually experienced WT males showed no decrement in sexual behavior 3 weeks after castration, while PRKO males had dramatic deficits in many measures, and naive males exhibited no sexual behavior at all. ANOVA revealed a significant influence of genotype, experience, and a genotype by experience interaction for mount latency and frequency measures ($P < 0.05$;

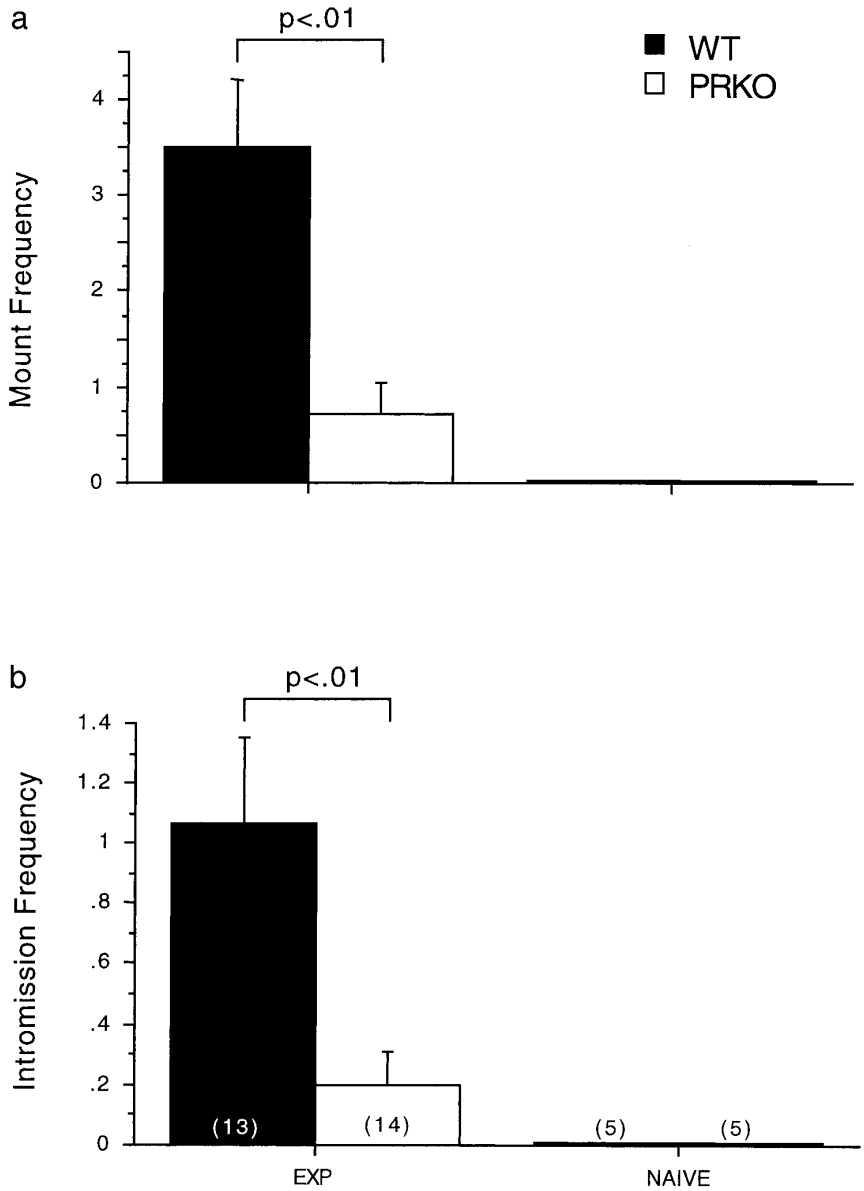

FIG. 1. Experience by genotype interactions in male sexual behaviors. (a) Mount frequencies of experienced (EXP) and naive (NAIVE) wild-type (WT) and progesterone receptor knockout (PRKO) males castrated for 3 weeks. (b) Intromission frequencies in 3-week castrates. Numbers beneath bars refer to sample sizes.

Fig. 1). Intromission latency and frequency scores exhibited significant experience effects ($P < 0.01$), but nonsignificant trends for both genotype and genotype by experience interactions ($P < 0.10$). Two-tailed t tests demonstrated that experienced, castrated PRKO males did indeed exhibit fewer intromissions and a higher latency to first intromission than identically treated WT males ($P < 0.01$). Ejaculation frequencies and latencies did not exhibit significant differences due to either experience or genotype ($P > 0.05$).

Study 2: Progesterone Receptor Deficits and Responses to Testosterone Replacement

Behavioral testing paradigm. All animals in the study were tested four times prior to castration and then once every 3 weeks for 9 weeks following castration. Males who were categorized as sexually responsive during intact tests, and who showed a decrement in two of three frequency measures following castration, were implanted and then tested 10 and 15 days following hormone treatment. This screening was

done to reduce variation in intact and castrate performances and facilitate detection of differential responses to hormone treatment.

All animals participating in the hormone study received two implants: the first was a testosterone implant (0.25 in. outer diameter, 0.062 in. inner diameter, and 5 mm in length, filled with crystalline T) or a size-matched capsule filled with silicone; the second was a progesterone implant (dimensions 0.085 in. outer diameter, 0.04 in. inner diameter, and 10 mm in length) or size-matched capsule filled with silicone. These dimensions have been demonstrated to produce physiological doses of plasma testosterone (Bronson, 1996). To reduce the rate of P diffusion into the plasma, we mixed 100 mg P with 1.0 ml of silicone and injected the mixture into Silastic tubing. The method has been used in mice in the past to deliver low doses of estrogen (Bronson, 1981) and physiological levels of plasma progesterone (L. J. Young and D. Crews, unpublished data).

Experienced animals from the first study were also subject to the same screening criteria and given hormone implants. There were not enough of these animals for a fully balanced study of the sort outlined here, so males were given either T or Bl capsules, in addition to a blank P-sized capsule. These data are presented because we feel they provide a more complete description of how PR may be interacting with plasma androgens to influence male sex behavior.

Statistical analysis. For the first four behavior tests (intact males), we averaged scores and performed ANOVAs with mount, intromission, and ejaculation frequencies as dependent variables and with genotype as the main effect. We performed repeated-measures ANOVAs for the three postcastration / preimplant tests (tests 5–7). Similar analyses were performed on latency data. To examine the influence of hormones, we performed an ANOVA on average postimplant frequency scores (tests 8–9), expressed as a percentage of precastration behaviors (average frequency scores are defined as the average of mount frequency (MF%), intromission frequency (IF%), and ejaculation frequency (EF%)) and one on latency scores subject to the same corrections. In this analysis, genotype, T treatments, and P treatments were the main effects. Because we made an *a priori* prediction of an interaction between genotype and hormone treatment, one-tailed *t* tests were performed to compare average frequency and latency scores between genotypes given the same hormone treatment. Where significant differences were found between latency or frequency averages of the two genotypes, we compared each behavior (mount, intromission, and ejaculation) between genotypes given the same hormone treatment. Because of time limits in the testing paradigm, ejaculation data are necessarily nonnormally distributed, so these comparisons were made with Mann–Whitney *U* tests.

Data from PRKO males of the first study, treated with T ($n = 10$) or blank capsules ($n = 3$), are included for completeness but were excluded from the statistical analysis.

Results. Analysis of preimplant frequency and latency behaviors revealed no significant influence of genotype (WT vs HTZ) on the behaviors of either intact or castrated males ($P > 0.05$). Intact wild-type males did, however, exhibit a nonsignificant trend ($P < 0.10$) toward a drop in mount latency relative to intact heterozygotes.

The three-factor ANOVAs revealed no influence of P treatment ($P > 0.05$) on either latency or frequency composite scores (average %), so animals given the same T treatment were pooled into a single group without respect to P treatment. The analysis was repeated as a genotype by testosterone treatment design. This analysis demonstrated that there were significant influences of testosterone ($P < 0.01$, frequency; $P < 0.05$, latency) as one would expect. The frequency analysis demonstrated a nonsignificant trend ($P < 0.10$) in the main effect for genotype and for genotype by testosterone interaction. Analysis within testosterone treatment revealed that wild-type males exhibited significantly more frequent sexual behaviors than heterozygotes (T-treated WT vs HTZ males, $P < 0.05$) and that this could not be attributed to any differences in the baseline of their behaviors (blank-treated WT vs HTZ males, $P > 0.05$) (Fig. 2). The clear absence of any influence of genotype in blank-treated males, as predicted, presumably underlies genotype's lack of significance in the gross analysis of frequency measures.

Analysis of component scores revealed that ejaculation frequencies were significantly higher in wild-type testosterone-treated males than in heterozygous testosterone-treated males ($P < 0.05$) and that intromission frequencies exhibited a trend ($P < 0.10$) toward being larger in T-treated WT males than in HTZ males (Table 1). There was no evidence for an influence of genotype in the average of latency scores.

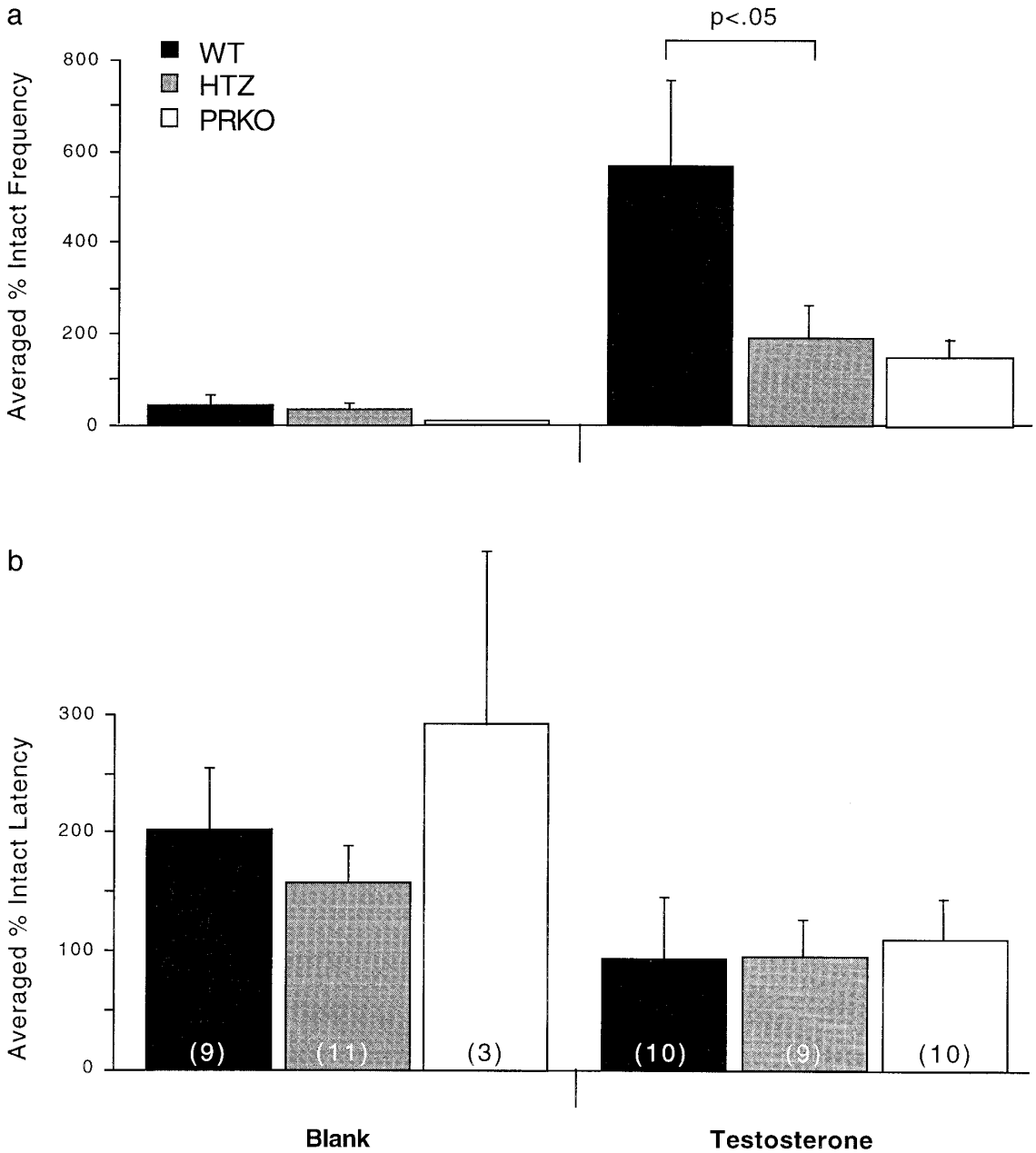

FIG. 2. (a) Averaged frequency of male-typical sexual behaviors. Values are expressed as a percentage of intact behaviors and averaged for the three frequency measures (mounts, intromissions, and ejaculation) for three genotypes, wild-type (WT), heterozygote (HTZ), and progesterone receptor knockout (PRKO) males. (b) Averaged latency of male typical sexual behaviors, expressed as a percentage of levels performed while intact. A two-way ANOVA revealed a significant influence of testosterone treatment ($P < 0.05$), but no influence of genotype ($P > 0.05$). Available data from progesterone receptor knockout (PRKO) males are included for completeness but were not subject to statistical analysis. Numbers in parentheses indicate sample sizes.

DISCUSSION

Results from the first study indicate that PRKO mice mount less often when naive, but this deficit is a modest one and can be alleviated by sexual experience. To our surprise, there are profound differences in the way the two genotypes react to castration when provided with prior experience. Although naive WT

and PRKO males have no sex behavior 3 weeks after castration, experienced WT males show no decline in their behaviors. Experienced, castrated PRKO males show some sexual behavior, but at much lower levels than the WT mice. We suggest that PR takes part in a pathway used by experience to bypass androgen dependence. This is a novel finding and deserves further consideration.

Researchers investigating male sexual behavior in rats have noted that experienced males displaying sexual behaviors following castration exhibit preceding surges of dopamine (DA) in the POA, while males that cease to respond following castration do not (Hull *et al.*, 1995). Subsequent analyses have revealed that POA infusions of the general dopaminergic agonist apomorphine, or the D1-receptor agonist SKF 38393, restore copulation to previously nonresponsive castrates (Hull *et al.*, in press). Recent studies have also demonstrated that DA is capable of eliciting female-typical behavior through the ligand-independent activation of PR in the ventromedial hypothalamus in female mice (Mani *et al.*, 1994, 1996). We propose that similar mechanisms operate in the POA to elicit male sexual behavior. More precisely, we hypothesize that experience potentiates preoptic DA release, which causes ligand-independent activation of PR and potentiation of male-typical sexual behavior. To address this hypothesis, we are currently investigating whether PRKO males are responsive to dopaminergic agonists and whether experienced WT castrates show a drop in sexual behavior when injected with dopamine antagonists.

The second study demonstrates that male mice heterozygous for a null mutation show impaired responses to T replacement therapy. The deficits appear to be restricted to frequency measures, particularly to ejaculation frequencies. Testosterone-treated PRKO males appear to share frequency deficits (Fig. 2a). These particular measures are sometimes regarded as more indicative of sexual performance than sexual motivation (Everitt, 1990; however, it is worth noting that not all "performance" measures changed by genotype). The POA is often considered the structure most directly influencing sexual performance in males (Everitt, 1990). Work on rats and lizards has demonstrated that microinjections or implantation of P into the POA facilitates androgen-induced sexual behavior (Witt *et al.*, 1997; Crews *et al.*, 1996), and the reptilian work has shown an increase in preoptic AR mRNA in response to systemic P treatment (Crews *et al.*, 1996). These data, though from seemingly disparate sources, are consistent with the hypothesis that PR and AR interactions in the medial POA may synergize to promote sexual behaviors in males. We cannot, of course, rule out the possibility that some or all of PR's influence is mediated through interactions with ER. Testosterone may influence sex-typical behaviors after being aromatized to estradiol (Sachs and Meisel, 1994), and ER–PR interactions are the norm in the regulation of female-typical behaviors. Moreover, although PR activity in the POA appears to be the most parsimonious explanation for the current data, we cannot exclude PR influences on other structures. The mechanisms proposed here are working hypotheses, and alternative explanations certainly merit additional study.

One superficial anomaly in our data is the apparent absence of an influence of exogenously administered P on male-typical sexual behavior. There are several possible explanations for this phenomenon. Most notable is the fact that native P—presumably of adrenal origin (Piva, Gagliano, Motta, and Martini, 1973)—has not been manipulated. If normal physiological levels of P are sufficient for PR activation, endogenous levels would need to be depleted before exogenous treatment could be expected to produce a measurable difference in sexual behaviors. Alternatively, PR may promote male sexual behavior through predominantly ligand-independent mechanisms, rendering native P unimportant in male mice. Studies of antiprogestins such as RU38486 should clarify the role of endogenous P in male sex behavior.

Given the large sample sizes used in the study of intact male behaviors, the normal behavior of intact HTZ males suggests that their genetic deficits can be compensated for by regulatory mechanisms at the level of receptor expression or steroid production or through other interacting loci. In this sense, the data are consistent with a wide array of studies on behaviors of knockout mice (Nelson, 1997). Researchers investigating estrogen receptor knockout mice, for example, did not find deficits in either mounting frequency or latency of intact males (Ogawa *et al.*, 1997), but other researchers have found clear deficiencies in these behaviors following castration and T replacement (Rissman, Wersiger, Taylor, and Lubahn, 1997; Wersiger, Sannen, Villalba, Lubahn, Rissman, and De Vries, 1997). Similarly, a recent report demonstrated that disruption of the steroid receptor coactivator-1 gene (SRC-1) does not impair the behavior of intact mice, but does reduce the response of mice to steroid replacement therapy (Xu, Qiu, DeMayo, Tsai, Tsai, and O'Malley, 1998). *In vitro* studies of receptor interactions indicate that PR is capable of serving as a

coactivator to modify the transcriptional activity of ER and AR in culture (Yen, Bai, Allgood, and Weigel, 1997). Whether such interactions regulate behaviors remains to be seen.

These data contribute to the growing body of literature indicating that the PR not only influences sexual behavior in males, but does so in diverse vertebrate species. Given that P appears to be capable of inducing either male-typical or female-typical sexual behavior in various species (Witt *et al.*, 1994; Young and Crews, 1995; Sachs and Meisel, 1994; Pfaff *et al.*, 1994; Mani *et al.*, 1997) and that there are clearly at least two isoforms of PR in taxa that have been investigated (Pham, Hwung, Santiso-Mere, McDonnell, and O'Malley, 1992; Vegeto, Shahbaz, Wen, Goldman, and O'Malley, 1993; Wen, Xu, Mais, Goldman, and McDonnell, 1994; Zhang, Bai, Allgood, and Weigel, 1994), it will be interesting to determine whether there are sexual dimorphisms in hypothalamic and preoptic expression profiles of these isoforms and whether such differences are related to sex-specific patterns of behavior. Perhaps such studies will prove that progesterone receptors, like androgen and estrogen receptors, are more androgynous than we anticipated.

ACKNOWLEDGMENTS

We thank Shirley Beckwith, Aaron Engels, Qouquing Ge, Jon Sakata, and Kira Wennstrom for technical assistance. Frank Bronson and Walt Wilczynski provided input on study design and presentation. This research was supported by MH F31 11194 (to S.P.), HD RO1 07857 (to B.W.O'M.), and MH R37 41770 and MH K05 00135 (to D.C.).

REFERENCES

Baum, M. J., Tobet, S. A., Starr, M. S., and Bradshaw, W. G. (1982). Implantation of dihydrotestosterone propionate into the lateral septum or medial amygdala facilitates copulation in castrated male rats given estradiol systemically. *Horm. Behav.* **16,** 208–223.

Beach, F. A., and Fowler, H. (1959). Individual differences in the response of male rats to androgen. *J. Comp. Physiol. Psychol.* **52,** 50–52.

Beach, F. A., and Holz-Tucker, A. M. (1949). Effects of different concentrations of androgen on sexual behavior in castrated male rats. *J. Comp. Physiol. Psychol.* **42,** 433–453.

Bottoni, L., Lucini, V., and Massa, R. (1985). Effect of progesterone on the sexual behavior of the male Japanese quail. *Gen. Comp. Endocrinol.* **57,** 345–351.

Bradford, J. M. W. (1988). Treatment of sexual offenders with cyproterone acetate. *In* J. M. A. Sitse (Ed.), *Handbook of Sexology, Vol. 6, The Pharmacology and Endocrinology of Sexual Function,* pp 526–536. Elsevier, New York.

Bronson, F. (1981). The regulation of luteinizing hormone secretion by estrogen: Relationships among negative feedback, surge potential, and male potential in juvenile, peripubertal and adult female mice. *Endocrinology* **108,** 506–516.

Bronson, F. (1996). Effects of prolonged exposure to anabolic steroids on the behavior of male and female mice. *Pharmacol. Biochem. Behav.* **53,** 329–334.

Christensen, L. W., and Clemens, L. G. (1974). Intrahypothalamic implants of testosterone or estradiol and resumption of masculine sexual behavior in long-term castrated male rats. *Endocrinology* **95,** 984–990.

Clark, A. S., Davis, L. A., and Roy, E. J. (1985). A possible physiological basis for the dud-stud phenomenon. *Horm. Behav.* **19,** 227–230.

Clemens, L. G., Wee, B. E. F., Weaver, D. R., Roy, E. J., Goldman, B. D., and Rakerd, B. (1988). Retention of masculine sexual behavior following castration in male B6D2F1 mice. *Physiol. Behav.* **42,** 69–76.

Crews, D., Godwin, J., Hartman, V., Grammer, M., Prediger, E., and Sheppherd, R. (1996). Intrahypothalamic implantation of progesterone in castrated male whiptail lizards (*Cnemidophorus inornatus*) elicits courtship and copulatory behavior and affects androgen receptor- and progesterone receptor-mRNA expression in the brain. *J. Neurosci.* **16,** 7347–7352.

Damassa, D. A., Smith, E. R., Tennent, B., and Davidson, J. M. (1977). The relationship between circulating testosterone levels and male sexual behavior in rats. *Horm. Behav.* **8,** 275–286.

Erickson, C. J., Bruder, R. H., Komisaruk, B. R., and Lehrman, D. S. (1967). Selective inhibition of androgen-induced behavior in male ring doves (*Streptopelia risoria*). *Endocrinology* **81,** 39–44.

Erpino, M. J. (1969). Hormonal control of courtship behavior in the pigeon (*Columba livia*). *Anim. Behav.* **1,** 401–405.

Erpino, M. J. (1973). Temporary inhibition by progesterone of sexual behavior in intact male mice. *Horm. Behav.* **4,** 335–339.

Everitt, B. (1990). Sexual motivation: A neural and behavioral analysis of the mechanisms underlying appetitive and copulatory responses of male rats. *Neurosci. Biobehav. Rev.* **14,** 219–232.

Hull, E. M., Du, J., Lorrain, D. S., and Matuszewich, L. (1997). Testosterone, preoptic dopamine, and copulation in male rats. *Brain Res. Bull.* **44,** 327–333.

Hull, E. M., Du, J., Lorrain, D. S., and Matuszewich, L. (1995). Extracellular dopamine in the medial preoptic area: Implications for sexual motivation and hormonal control of copulation. *J. Neurosci.* **15,** 7465–7471.

Kage, A., Fenner, A., Weber, B., and Schoneshofer, M. (1982). Diurnal and ultradian variations of plasma concentrations of eleven adrenal steroid hormones in human males. *Klin. Wochenschr.* **60,** 659–666.

Kalra, P. S., and Kalra, S. P. (1977). Circadian periodicities of serum androgens, progesterone, gonadotropins and luteinizing hormone-releasing hormone in male rats: The effects of hypothalamic deafferentation, castration and adrenalectomy. *Endocrinology* **10,** 1821–1827.

Lehne, G. K. (1988). Treatment of sex offenders with medroxyprogesterone acetate. *In* J. M. A. Sitse (Ed.) *Handbook of Sexology, Vol. 6, The Pharmacology and Endocrinology of Sexual Function,* pp 516–25. Elsevier, New York.

Lindzey, J., and Crews, D. (1986). Hormonal control of courtship and copulatory behavior in male *Cnemidophorus inornatus,* a direct sexual ancestor of a unisexual, parthenogenetic lizard. *Gen. Comp. Endocrinol.* **64,** 411–418.

Lindzey, J., and Crews, D. (1988). Effects of progestins on sexual

behaviour in castrated lizards (*Cnemidophorus inornatus*). *J. Endocrinol.* **119,** 265–273.

Lindzey, J., and Crews, D. (1992). Interactions between progesterone and androgens in the stimulation of sex behaviors in male little striped whiptail lizards, *Cnemidophorus inornatus. Gen. Comp. Endocrinol.* **86,** 52–58.

Lydon, J. P., DeMayo, F. J., Funk, C. R., Mani, S. K., Hughes, A. R., Montgomery, C. A., Jr., Shyamala, G., Conneely, O. M., and O'Malley, B. W. (1995). Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev.* **9,** 2266–2278.

Mani, S. K., Allen, J. M. C., Clark, J. H., Blaustein, J. D., and O'Malley, B. W. (1994). Convergent pathways for steroid hormone and neurotransmitter-induced rat sexual behavior. *Science* **265,** 1246–1249.

Mani, S., Blaustein, J., and O'Malley, B. W. (1997). Progesterone receptor function from a behavioral perspective. *Horm. Behav.* **31,** 244–255.

McGill, T. E. (1965). Studies of the sexual behavior of male laboratory mice: Effects of genotype, recovery of sex drive, and theory. *In* F. A. Beach (Ed.), *Sex and Behavior,* pp. 76–88. Wiley, New York.

Nelson, R. J. (1997). The use of genetic "knockout" mice in behavioral endocrinology research. *Horm. Behav.* **31,** 188–196.

Ogawa, S., LuBahn, D., Korach, K., and Pfaff, D. W. (1997). Behavioral effects of estrogen receptor gene disruption in male mice. *Proc. Natl. Acad. Sci. USA* **94,** 1476–1481.

Opstad, K. (1994). Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep, and energy deficiency in young men. *Eur. J. Endocrinol.* **13,** 56–66.

Pfaff, D. W., Schwartz-Giblin, S., McCarthy, M. M., and Kow, L-M. (1994). Cellular and molecular mechanisms of female reproductive behaviors. *In* J. D. Neill and E. Knobil (Eds.), *The Physiology of Reproduction,* Vol. 2, pp 107–220. Raven Press, New York.

Pham, T., Hwung, Y., Santiso-Mere, D., McDonnell, D., and O'Malley, B. W. (1992). Ligand-dependent and independent function of the transactivation regions of the human estrogen receptor in yeast. *Mol. Endocrinol.* **6,** 1043–1050.

Piva, F., Gagliano, P., Motta, M., and Martini, L. (1973). Adrenal progesterone: Factors controlling secretion. *Endocrinology* **93,** 1178–1184.

Rissman, E. F., Wersinger, S. R., Taylor, J. A., and Lubahn, D. B. (1997). Estrogen receptor function as revealed by knockout studies: Neuroendocrine and behavioral aspects. *Horm. Behav.* **31,** 232–243.

Sachs, B., and Meisel, R. L. (1994). The physiology of male sexual behavior. *In* J. D. Neill and E. Knobil (Eds.), *The Physiology of Reproduction,* Vol. 2, pp. 3–106. Raven Press, New York.

Vegeto, E., Shahbaz, D., Wen, D., Goldman, M., and O'Malley, B. W. (1993). Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. *Mol. Endocrinol.* **75,** 1244–1255.

Vermueulen, A., and Verdonck, L. (1976). Radioimmunoassay of 17β-hyroxy-5α-androstan-3-one, 4-androstene-3, 17-dione, dehydroepiandrosterone, 17-hydroxyprogesterone and progesterone and its application to human male plasma. *J. Steroid Biochem.* **7,** 1–10.

Wagner, C. K., Nakayama, A. Y., and De Vries, G. J. (1998). Potential role of maternal progesterone in the sexual differentiation of the brain. *Endocrinology* **139,** 3658–3661.

Wen, D., Xu, Y., Mais, D., Goldman, M., and McDonnell, D. (1994). The A and B forms of the progesterone receptor operate through distinct signaling pathways within target cells. *Mol. Cell. Biol.* **14,** 8356–8364.

Wersinger, S. R., Sannen, K., Villalba, C., Lubahn, D. B., Rissman, E. F., and De Vries, G. J. (1997). Masculine sexual behavior is disrupted in male and female mice lacking a functional estrogen receptor α gene. *Horm. Behav.* **32,** 176–183.

Witt, D. M., Reigada, L. C., and Wengroff, B. E. (1997). Intrahypothalamic progesterone regulates androgen-dependent sexual behavior in male rats. *Soc. Neurosci. Abstr.* **23,** 1357. [Abstract 532.16]

Witt, D., Young, L., and Crews, D. (1994). Progesterone and sexual behavior in males. *Psychoneuroendocrinology* **19,** 553–562.

Witt, D., Young, L., and Crews, D. (1995). Progesterone modulation of androgen-dependent sexual behavior in male rats. *Physiol. Behav.* **57,** 307–313.

Xu, J., Qiu, Y., DeMayo, F. J, Tsai, S. Y., Tsai, M.-J., and O'Malley, B. W. (1998). Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. *Science* **279,** 1922–1925.

Young, L. J., and Crews, D. (1995). Comparative neuroendocrinology of steroid receptor gene expression and regulation: Relationship to physiology and behavior. *Trends Endocr. Metab.* **6,** 317–323.

Young, L. J., Greenberg, N., and Crews, D. (1991). The effects of progesterone on sexual behavior in male green anole lizards (*Anolis carolinensis*). *Horm. Behav.* **25,** 477–488.

Yen, P. M., Liu, Y., Palvimo, J. J, Trifiro, M., Whang, J., Pinksy, L., Janne, O. A., and Chin, W. W. (1997). Mutant and wild-type androgen receptors exhibit cross talk on androgen-, glucocorticoid-, and progesterone-mediated transcription. *Mol. Endocrinol.* **11,** 162–171.

Zhang, Y., Bai, W., Allgood, V., and Weigel, N. (1994). Multiple signaling pathways activate the chicken progesterone receptor. *Mol. Endocrinol.* **8,** 577–584.