Progesterone Receptors and the Sexual Differentiation of the Medial Preoptic Nucleus

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ABSTRACT: **The central component of the medial preoptic nucleus (MPNc) of the rat has served as an excellent model of sexual differentiation. The MPNc is larger in adult males than in females, and its development is regulated by perinatal gonadal hormones. Although testosterone (T) and its metabolite estradiol (E) sexually differentiate this region, the exact mechanism by which they act during development is not known. There is a dramatic sex difference in the expression of progesterone receptors (PR) in the MPN during development; perinatal males express higher levels of PR than females. Additionally, PR expression during this time is dependent on exposure to T. Thus, PR induction may be one mechanism by which T sexually differentiates the MPN. The present study investigated the potential role of PR in the sexual differentiation of the MPNc. Anatomical examination of PR distribution within the MPN of neonatal males revealed the presence of PR immunoreactive cells within the MPNc, suggesting a direct route of action for PR in the development of the MPNc. Additionally, we measured the effects of neonatal RU486 treatment, a progesterone and glucocorticoid receptor antagonist, on subsequent MPNc volume in neonatally T-treated females and neonatally castrated males, given T. RU486 treatment reduced the MPNc volume of T-treated females while it increased the volume in T-treated, neonatally castrated males. These results, taken together with the expression of PR in the MPNc, suggest that PR may influence the sexual differentiation of the MPNc volume.** © 2002 Wiley Periodicals, Inc. J Neurobiol 51: 24–32, 2002

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INTRODUCTION

Many behaviors in mammals are sexually dimorphic and presumably are a reflection of sex differences in the brain (for review, see Arnold and Gorski, 1984; De Vries, 1990; Breedlove, 1992). The central component of the medial preoptic nucleus (MPNc) of the rat brain has served as a model for studying the mechanisms involved in sexual differentiation. Originally coined the sexually dimorphic nucleus of the preoptic area (SDN-POA) (Gorski et al., 1978), the volume of the MPNc in the adult male is several times larger than in the adult female (Gorski et al., 1980; Davis et al., 1996). The sex difference in MPNc volume is attributable to the differential exposure of males and females to the gonadal steroid hormone testosterone (T) and its subsequent aromatization to estradiol (E) during specific periods of development.

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Adult females that were exposed perinatally to testosterone propionate (TP) or the synthetic estrogen diethylstilbestrol (DES) had a significantly larger MPNc volume than controls (Döhler et al., 1984; Rhees et al., 1990a, 1990b; Faber and Hughes, 1991). Similarly, adult males that were castrated on the day of birth or administered the aromatase inhibitor ATD perinatally had a reduced MPNc volume (Houtsmuller et al., 1994; Davis et al., 1996). In addition, there is a specific developmental period during which this region is most sensitive to hormonal manipulations; it spans the days between embryonic day (ED) 18 and postnatal day (PD) 5 (Rhees et al., 1990a, 1990b).

T exposure during perinatal development alters MPNc volume, in part, by decreasing the incidence of apoptosis or programmed cell death (Davis et al., 1996; Chung et al., 2000). Exposure of neonatal females to TP reduced cell loss in the MPNc (Dodson and Gorski, 1993). Similarly, castration of neonatal males increases the incidence of apoptosis unless males are treated with TP (Davis et al., 1996; Chung et al., 2000). However, the mechanisms underlying the ability of T to increase the cell survival in the MPNc of males are not well understood.

In the developing MPN, there is a dramatic sex difference in the expression of progesterone receptors (PR). Perinatal males express much higher levels of PR immunoreactivity (PRir) in the MPN than females (Wagner et al., 1998a). PR is first expressed in the MPN of fetal males on ED19 (Wagner et al., 2000)—around the time of the surge in circulating T levels (Weisz and Ward, 1980). The sex difference in PR expression continues until PD10, after which time females begin to express PR in the MPN (unpublished observations), thereby reducing the magnitude of the sex difference. The induction of PR in the MPN of developing males appears to be dependent on the conversion of T to E. Prenatal TP or DES induced PR expression in the MPN of females and abolished the sex difference normally present on the day of birth (Wagner et al., 1998b), and neonatal TP treatment similarly induced PR in the MPN of PD3 females (Quadros and Wagner, unpublished observations). In addition, PR expression in the MPN is substantially reduced in neonatal male mice lacking a functional estrogen receptor alpha (ERα) gene (Wagner et al., 2001), suggesting that PR expression in the developing male MPN is produced by E acting through the alpha isoform of the estrogen receptor.

Because T induces PR expression in the MPN during development and the masculinization of the MPNc is dependent on T, the possibility exists that this hormone alters MPN development, at least in part, through the induction of PR. To determine if PR could directly influence the development of the MPN, we first examined the distribution of PR within the subdivision of the MPN in PD4 males. PR expression appeared to be present in the MPNc in a rostral-to-caudal gradient. Additionally, to assess the contributions of PR function on the sexual differentiation of the MPNc, we blocked PR activity with the progesterone and glucocorticoid receptor (GR) antagonist, RU486, in TP-treated castrated males and TP-treated females during early postnatal development. Findings from these experiments implicate PR as a modulator of sexual differentiation of the MPNc.

METHODS

Animals

All the animals in this study were maintained in accordance with protocols approved by the Institutional Animal Care and Use Committee at the University of Massachusetts, Amherst. Eighteen female virgin Sprague-Dawley rats (65–80 days of age, Taconic Farms, Germantown, NY) were mated with sexually experienced Sprague-Dawley stud males from our colony. After mating, pregnant females were housed in hanging wire cages in groups of two until the last week of their pregnancy, at which point they were housed in individual polypropylene cages and left undisturbed until the day of parturition. Pregnant and lactating females and their pups were all maintained in a temperature- (22°C) and light-controlled room (14L:10D, lights on at 0500 h) with food and water available *ad libitum*.

Experiment 1: PR Expression in the MPNc

Neonatal Injections. Four male neonates were obtained from litters produced by three adult females mated as described above. Because these males represented the control group of a larger study examining the induction of PR by testosterone they received 0.05 cc of the sesame oil vehicle from the day of birth until the day of sacrifice (PD4).

Tissue Preparation. Subjects were sacrificed on PD4 by decapitation after anesthesia by cooling them on ice. Brains were removed and immersion-fixed in 5% acrolein in 0.1 *M* phosphate buffer (PB, pH 7.6) overnight, followed by cryoprotection in 30% sucrose in 0.1 *M* PB. Brains were cut at 40 μm thickness on a sliding microtome and stored in cryoprotectant (30% sucrose, 0.1% polyvinyl-pyrrolidone-40 in ethylene glycol and 0.1 *M* PB) at −20°C until processing.

Immunocytochemistry and Nissl Stain. Immunocytochemistry for PRir was performed as described previously (Wagner et al.,1998a). Briefly, free-floating sections were

Table 1 Total Number of Animals (Litters) Represented in Each Treatment Group

	Experiment 2: Females		Experiment 3: Males
	Testosterone Propionate	Oil	Testosterone Propionate
RU486	$N = 10$ (5 litters)	$N = 9$ (4 litters)	$N = 7$ (5 litters)
Oil	$N = 11$ (3 litters)	$N = 11$ (3 litters)	$N = 10$ (3 litters)

rinsed with 0.05 *M* Tris-buffered saline (TBS, pH 7.6) and incubated in 1% sodium borohydride in TBS for 10 min. Sections were rinsed in TBS followed by incubation in normal serum (1% H_2O_2, 20% normal goat serum, and 1% bovine serum albumin in TBS) for 20 min. Sections were incubated for 72 h in rabbit polyclonal antisera to PR (1:1000; DAKO, Inc., Glostrup, Denmark), that recognizes both the A and B forms of the receptor (Traish and Wotiz, 1990). Sections were rinsed with TBS containing 0.3% Triton-X 100 and 2% normal goat serum and incubated in biotinylated goat antirabbit IgG (1:200; Vector Laboratories, Burlingame, CA) for 1 h. Following four rinses, sections were incubated in ABC (Vetastain Elite Kit; Vector Laboratories, Burlingame, CA) for 1 h, rinsed, and then incubated in TBS containing 0.05% diaminobenzidine, 0.75 m*M* nickel ammonium sulfate, 0.15% β-D glucose, 0.04% ammonium chloride and 0.001% glucose oxidase for approximately 20 min. Sections were rinsed, mounted onto gelatin-coated slides, and allowed to air dry. Slides were rinsed in distilled water and stained with thionin [0.25% Thionin (Sigma, Inc.)] in 0.2% glacial acetic acid and 0.7% sodium acetate for approximately 3–5 min. Slides were then rinsed in water, dehydrated in a series of alcohols, delipidated with Hemo-D (Fisher Scientific), and coverslipped with Permount (Fisher Scientific).

Experiment 2: TP-Treated Females

Neonatal Injections. Forty-one TP-treated Sprague-Dawley females, the offspring of pregnant females that were obtained as described earlier, were used as subjects in this experiment. Previous studies have used TP-treated females as a model to investigate the mechanisms underlying sexual differentiation and the role of T in masculinization of the brain (Simerly et al., 1985; Dodson and Gorski, 1993). Female pups received injections of either TP (100 μg/0.05 cc sesame oil, s.c.), or an equal volume of sesame oil vehicle, daily from PD1 until PD21, the day of sacrifice. This dose of TP significantly masculinized the MPNc volume of castrated males or intact females to a volume similar to that of intact control males (Rhees et al., 1990b; Davis et al., 1996).

Although the sex difference in PR expression is first present prenatally, it is not feasible to manipulate PR function *in utero* because progesterone is necessary to maintain pregnancy. Therefore, antagonist treatment in the present study began on the day of birth. Roughly half of the females in each of the TP and oil groups received either RU486 (mifepristone; 17α-hydroxy-11[4-dimethyl-aminophenyl] 17-propenyl-estra-4, 5-diene-3-one (Sigma, Inc.) at a dose of 20 μg/0.01 cc/g B.W., s.c.) (TP+RU486; oil+RU486) or an equal volume of oil vehicle (TP+oil; oil+oil) daily from PD1 through PD8, a time during which the sex difference in PR expression in the MPN is most dramatic. Preliminary studies demonstrate that this dose of RU486 blocks PR-dependent female sexual behavior in estradiol-primed adult females, confirming that this dose is centrally active (unpublished observations). These treatments generated four groups summarized in Table 1.

Experiment 3: TP-Treated Castrated Males

Neonatal Castration. Seventeen Sprague-Dawley males, the offspring of pregnant females that were obtained as described earlier, were used as subjects in this experiment. To control for any indirect effects RU486 may have on T secretion, males were castrated on the day of birth, as described below, and given exogenous TP (100 μg/0.05 cc/day). Within 18 h of birth, males were anesthetized by cooling them on ice. A midline incision was made in the abdomen just below the umbilical cord with the aid of a dissecting microscope. Both testes were removed with a fine surgical forcep. The abdominal incision was sealed with Collodion™ (Sigma, Inc.), a nitrocellulose solution containing 4% pyroxylin, in 75% ether and 25% ethanol. Following surgery, pups were warmed on a heating pad, under a lamp and allowed to recover for at least 2 h before being returned to their mothers, after which they were carefully monitored.

Neonatal Injections. Roughly half of the TP-treated castrated males received RU486 (TP+RU486) or the oil vehicle (TP+oil) as in Experiment 2. These treatments generated two groups, which are summarized in Table 1.

Tissue Preparation. In Experiment 2, all females received their last treatment of either TP or oil on PD21, while in Experiment 3 all males received their last treatment of TP on PD21. Two to four hours postinjection, animals were given a lethal dose of chlorapent (0.25 *M* chloral hydrate, 0.08 *M* magnesium sulfate, 45 m*M* pentobarbital, 3 *M* ethyl alcohol, 4.5 *M* propylene glycol in distilled water), and were intracardially perfused with approximately 100 mL of 0.9% saline followed by 250 mL of 10% buffered formalin (Fisher Scientific). Brains were immediately removed from the skulls and postfixed in formalin for 2 h followed by immersion in 30% sucrose in 0.1 *M* PB (pH 7.6) at 4°C, for at least

24 h prior to sectioning. Brains were blocked, frozen, and serial coronal sections through the entire rostral–caudal extent of the MPNc were cut at 40 μm thickness on a sliding microtome into 0.05 *M* TBS (pH 7.6) and immediately mounted onto gelatin-coated slides. Tissue sections were allowed to air dry at room temperature and were stained with cresyl violet (pH 3.5), dehydrated, and coverslipped with Permount, as described in Experiment 1.

Morphometric and Statistical Analyses (for Experiments 2 and 3)

The MPNc was delineated using specific criteria of cell density, cell orientation, and intensity of Nissl staining, which were based on the divisions of the MPN determined by serotonergic innervation and originally described by Simerly et al. (1984). In rostral sections containing the MPNc, we used cell orientation as the criteria for the outer borders of the MPNc. In the caudal sections, however, we used cell density to distinguish the MPNc from the lateral and medial divisions (Simerly et al., 1984). All serial sections on one side of the brain containing the MPNc were analyzed by two independent observers, blind to the treatment groups. Boundaries were drawn to include the cells that fit the above-mentioned criterion using a computer-assisted image analysis system and the NIH Image program. Microscopic images of the MPNc were captured with an Olympus BH-2 microscope fitted with a CCD72 camera (Dage, MTI, Michigan City, IN) attached to a Quick Capture frame grabber board (Data Translation, Marlboro, MA) in a Macintosh IIFx computer. NIH Image software (W. Rasband, National Institutes of Health, Bethesda, MD) was used to measure captured images of the MPNc. The area of the MPNc for each section was determined (pixels) and converted to μm^2 using a conversion factor determined with a slide micrometer. The total volume (μm^3) of the MPNc for each animal was calculated as the summation of the serial cross-sectional areas (μm^2), multiplied by section thickness [Volume (μm^3) = Σ (Area μm^2) $\times$ (40 μm)]. The total volume of one side of the MPNc for each animal from each observer was calculated as the average of the two independent observers' measurements. These values were used in statistical analysis. The tissues from Experiment 2 and 3 were analyzed independently.

For Experiment 2, all treatment groups were analyzed using a 2 $\times$ 2 Analysis of Variance (ANOVA) (hormone $\times$ antagonist treatment). Preplanned multiple comparisons were made using Student-Newman-Keuls test ($p < 0.05$). In Experiment 3, the two groups were compared using Student's *t*-test.

RESULTS

Experiment 1

Within each oil-treated male, approximately three to four serial 40 μm coronal sections contained the MPNc. PRir was present in the MPNc but at lower levels than in other subdivisions [Fig. 1(A)]. Additionally, PRir distribution in all regions of the MPN followed a rostral-to-caudal gradient, i.e., rostral sections of the MPNc appear to have relatively more cells positive for PRir than caudal sections.

Experiment 2

Neonatal RU486 treatment attenuated the masculinizing effects of TP on the MPNc volume in females (Fig. 2). Two-way ANOVA revealed significant main effects of hormone treatment, $F(1, 37) = 107.21$, $p < 0.0001$, and antagonist treatment, $F(1, 37) = 4.98$, $p < 0.05$. Additionally, a significant hormone by antagonist interaction, $F(1, 37) = 5.32$, $p < 0.05$.

Post hoc analysis revealed that TP+oil treated females had a significantly larger MPNc volume compared to oil+oil ($p < 0.05$). Additionally, TP+RU486-treated females had a significantly smaller MPNc volume compared to TP+oil-treated females ($p < 0.05$). There were no significant differences between oil+oil and oil+RU486 females.

Experiment 3

Neonatal RU486 treatment increased the MPNc volume in castrated males given TP. TP+RU486-castrated males had a significantly larger MPNc volume than the TP+oil-castrated controls ($p < 0.05$) (Fig. 3).

DISCUSSION

The purpose of this study was to examine the role of PR in the sexual differentiation of the MPN. Because both PR expression in the developing MPN and the masculinization of the MPNc are dependent on T, we hypothesized that PR induction might be a mechanism by which T sexually differentiates the MPN. Upon examining the distribution of PR, we observed a rostral-to-caudal gradient of expression within the MPN and MPNc of PD4 males. Additionally, treatment with RU486 during early postnatal development significantly altered the volume of the MPNc in both males and TP-treated females; RU486 decreased MPNc volume in TP-treated females while it increased MPNc volume in castrated males given exogenous TP. Together, these results suggest a role for PR in the sexual differentiation of the MPN.

The presence of PRir within the MPNc of neonatal animals suggests that PR may act directly on MPNc cells to influence its organization. However, PR ex-

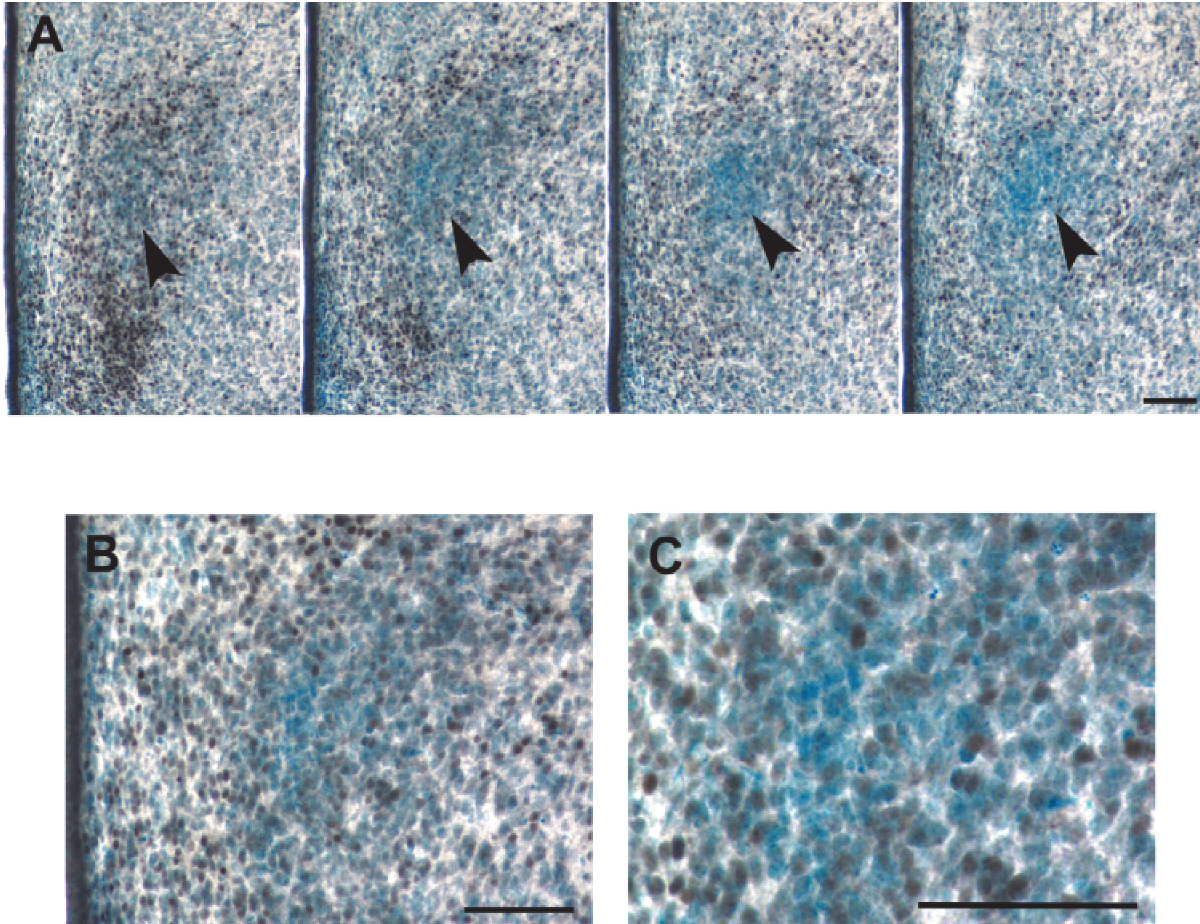

Figure 1 Coronal 40-μm sections through the central component of the medial preoptic nucleus (MPNc; arrowhead) that had been processed for immunocytochemistry using an antisera against progesterone receptor (PR) (black nuclear stain) and have been counterstained with Nissl stain (blue). (A) Serial sections through the rostral–caudal extent of the MPNc of a representative male at postnatal day 4. (B and C) PR immunoreactive cells in the MPNc of a postnatal day 4 male at higher maginification. Bars = 100 μm.

pression in the MPNc is relatively low compared to the surrounding regions of the MPN. It appears that a greater proportion of the cells that were positive for PRir in the other subdivisions of the MPN compared to the MPNc. Like the MPNc, there is a sex difference in the size of another subdivision of the MPN, MPNl, which is also influenced by perinatal T exposure (Simerly et al., 1984, 1985). Although we did not systematically examine the distribution of PRir in the MPN, it is also possible that higher levels of T-induced PRir in other subdivisions of the MPN could indirectly influence the development of the MPNc. Within the MPNc, we systematically observed a rostral-to-caudal gradient in the distribution of PR such that a greater number of PR-immunoreactive cells were present in more rostral MPNc sections compared to caudal ones. Although no information is available on rostral-to-caudal variability in function, chemical phenotype, or connectivity of the MPNc, the distribution of PR in the developing MPNc suggests that it may influence rostral and caudal regions of the MPNc differently.

In addition to PR, other steroid receptors are expressed in the developing MPN in a sexually dimorphic manner. Estrogen receptor alpha (ERα) is present in the MPN during prenatal and postnatal development. Females express higher levels of ERα than males (DonCarlos and Handa, 1994; DonCarlos, 1996; Yokosuka et al., 1997), due to the fact that estradiol, a ligand that is present at higher levels in males, downregulates the expression of ERα (Lauber et al., 1991; Simerly and Young, 1991; Balustein, 1993; Brown et al., 1996). Like the expression of PR, ERα immunoreactivity is much lower in the MPNc

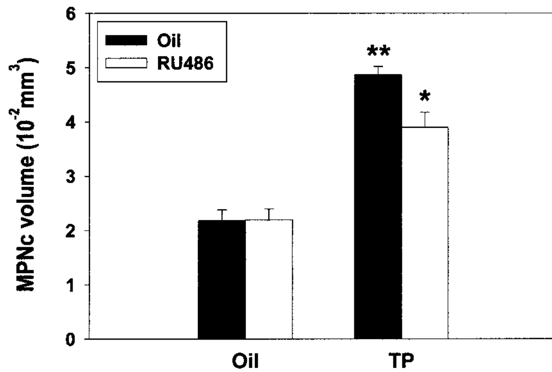

Figure 2 The volume (10^{-2} mm^3) of the central component of the medial preoptic nucleus (MPNc) in postnatal day 21 females that received testosterone propionate or oil vehicle on postnatal days 1–21 and either the PR antagonist, RU486, or oil on postnatal days 1–8. *Significantly different from TP+oil females ($p < 0.05$); **significantly different from oil+oil-treated females ($p < 0.001$). No significant differences were found between oil+oil and oil+RU486 females.

compared to the surrounding subdivisions of the MPN in postnatal animals (Yokosuka et al., 1997), consistent with the idea that PR expression in the MPN and MPNc during development is induced by genomic actions of ERα. Although AR expression is also sexually dimorphic in the MPN during this time, with males expressing higher levels of AR than females (McAbee and DonCarlos, 1998), its specific distribution within the MPNc has not been reported.

In the present study, we administered RU486 to neonatal females treated with TP and examined the effect of this treatment on subsequent MPNc volume. Neonatal administration of RU486 decreased the MPNc volume of TP-treated females but had no effect on oil-treated controls. Although RU486 is both a PR and a GR antagonist, there are several lines of evidence to suggest that the effects of RU486 in this study were mediated by PR and not GR. With the exception of the paraventricular nucleus and suprachiasmatic nucleus, GR mRNA and protein levels are low in the hypothalamus during the first week of life compared to other regions of the developing brain (Rosenfeld et al., 1988; van Eekelen et al., 1991; Yi et al., 1994). Additionally, unlike PR expression, there is currently no evidence suggesting a relationship between T or E and GR expression during development. In contrast, T induces PR expression in females, which fits well with the effects of RU486 on MPNc volume in females, i.e., RU486 treatment decreased the MPNc volume of TP-treated females but had no effect on the MPNc volume of control (non-TP) females. Finally, during the first 2 weeks of life, the time period during which RU486 was administered, the hypothalamic–pituitary–adrenal (HPA) axis is hyporesponsive to stress and stress-related manipulations (for review, see Vazquez, 1998). Taken together, these findings make it unlikely that GR was the receptor responsible for the effects of RU486 on MPNc volume and implicate a role for PR in the sexual differentiation of the MPN. While we observed a significant reduction in the MPNc volume of TP-treated females, RU486 did not completely reverse the effects of T. This may be an effect of the dose or timing of RU486 treatment or the existence of alternative mechanisms by which T alters the development of the MPNc, in addition to PR induction.

Neonatal treatment with RU486 increased the MPNc volume of castrated males given TP. To our knowledge only one other study has reported an increase in MPNc volume following neonatal treatment. The MPNc volume of adult animals that had been neonatally treated with a β2-adrenergic receptor agonist was larger compared to controls (Jarbaz et al., 1990). Although the MPNc has not been directly implicated in mediating any sexually dimorphic behavior in particular, the MPN has been shown to regulate male sexual and maternal behaviors (Meisel and Sachs, 1994; Numan, 1994). Recently, our laboratory reported reductions in male sexual behavior in adult males that had been treated with RU486 as neonates. Although RU486 treatment did not alter circulating T levels in neonatal males, the number of males displaying mounts, intromissions, and ejaculations was significantly lower in RU486-treated males compared to controls (Lonstein et al., 2001). This finding, taken together with the findings from Experiment 3, is consistent with the idea that the sex dif-

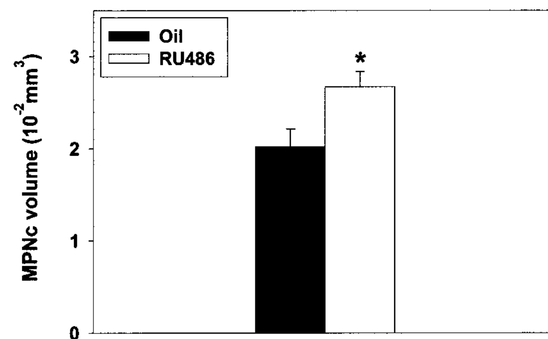

Figure 3 The volume (mm^3) of the central component of the medial preoptic nucleus (MPNc) in postnatal day 21 males that were castrated on the day of birth and received testosterone propionate on postnatal days 1–21 and either the progesterone receptor antagonist, RU486, or the oil vehicle on postnatal days 1–8. *Significantly different from oil-treated males ($p < 0.05$).

ference in PR expression in the developing MPN may influence the sexual differentiation of the structure and function of the MPN.

The discrepancy in the effects of RU486 on the development of the MPNc volume in TP-treated males and females, with RU486 decreasing the MPNc volume in females but increasing it in males, may be the result of several factors, the most parsimonious of which is the presence of sex differences in prenatal hormonal environment. The MPNc (reported as the SDN-POA) is already sexually dimorphic on the day of birth, although not to the degree to which it is observed in adulthood (Jacobson et al., 1980), presumably due to high levels of T secreted by the fetal testes. A single injection of TP before birth is sufficient to increase MPNc volume in females compared to control females (Rhees et al., 1990a), suggesting that although the MPNc undergoes significant differentiation postnatally, prenatal hormone exposure alone can significantly alter the development of the MPNc. Additionally, the sex difference in PR expression is present as early as ED19. The males in this study presumably had functional PR in the MPN 4 days *in utero*, prior to the onset of RU486 treatment. Therefore, the MPN may be sufficiently differentiated by the day of birth, such that the response to RU486 differs in males and females even in the presence of comparable postnatal hormone treatment. We can only speculate on the cellular and molecular basis for the prenatal hormone environment to produce opposite responses to PR inhibition neonatally. Prenatal testosterone exposure might alter the population of cells occupying the MPNc by the day of birth by altering neurogenesis, cell migration, or neuronal phenotype. These results suggest that neonatal females treated with testosterone and neonatal males may not be identical models of sexual differentiation.

Although differences in prenatal hormones is the most dramatic difference between males and females in this study, another factor for consideration is that the females in this study were gonadally intact. Although much of the literature on sexual differentiation has attributed the development of sex differences in the central nervous system to testosterone, the ovaries have been shown to contribute subtly to the sexual differentiation of both the brain and behavior (see Fitch and Deneberg, 1998). It is possible that ovarian factors, secreted from the ovaries of the intact females in this study, may have played a role in the sexually dimorphic response to RU486. However, the masculinizing effects of T in females seem to override any ovarian influence in almost all sexually differentiated neural systems. In addition, an effect of RU486 was seen only in the presence of TP in the present study. This makes a direct interaction between ovarian factors and RU486 an unlikely explanation for the differential response to RU486 in males and females receiving identical postnatal treatment.

Although the mechanisms by which PR alters MPNc volume are not known at present, T masculinizes the MPNc volume by altering the incidence of apoptosis during early postnatal development (Davis et al., 1996). Because apoptosis was not measured in the current study, we can only suggest by inference that RU486 altered the rate of cell death in the MPNc. However, several lines of evidence suggest that progesterone may regulate the molecular mechanisms involved in apoptosis. Interestingly, progesterone can either induce or inhibit apoptosis depending on cell type and treatment. For example, progesterone increased the levels of bcl-X_L, a member of the bcl-2 family of pro-life proteins (for review, see Merry and Korsmeyer, 1997) in endometrial cells and thereby prevented apoptosis (Pecci et al., 1997). In contrast, progesterone treatment, in conjunction with estradiol, reduced bcl-2 levels in neurons. Estradiol upregulated bcl-2 expression in the neurons of the arcuate nucleus in rats, but progesterone reduced estradiol's effectiveness in increasing bcl-2 expression (Garcia-Segura et al., 1998). Although the differential effect of progesterone on apoptosis has been demonstrated in different cell types, it is possible that such an effect might occur *in vivo* in the developing brain. Steroid receptors are powerful transcription factors that have the ability to permanently alter gene expression. Thus, it is possible that the exposure to prenatal T could have rendered the population of cells in the MPN of neonatal males different (e.g., with regard to their neurochemical phenotype) such that responses to similar drug treatment could result in altered rates of apoptosis. In this manner, it is possible for progesterone to have a dual role in apoptosis; through different pathways, progesterone could induce apoptosis in some cell populations while promoting cell survival in others.

While T from the fetal and neonatal testes appears to be the principal director of sexual differentiation, nontesticular factors, in particular progesterone, may play an important modulatory role in this process. In the present study, neonatal RU486 treatment significantly altered the volume of the MPNc in both males and TP-treated females, implicating a role for PR in the development of sex differences in the MPN. TP-treated females and castrated males responded differently to similar treatment of RU486. These findings are suggestive of complex mechanistic and temporal interactions between PR function and the actions of T that may occur during critical periods of neural de-

velopment producing sex differences in brain structure and function.

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