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# Neuroendocrine Regulation of Sexually Dimorphic Brain Structure and Associated Sexual Behavior in Male Rats Is Genetically Controlled[1]

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## ABSTRACT

**Steroid hormones, particularly 17β-estradiol ($E_2$), regulate the development and expression of neural structures and sexual behavior. Recently, we demonstrated that $E_2$-regulated responses are controlled by quantitative trait loci. In this study, we quantified 1) volume of the sexually dimorphic nucleus (SDN) of the preoptic area (POA); 2) medial basal hypothalamic (MBH)-POA aromatase and 5α-reductase enzyme activities during prenatal development and in adults; 3) serum LH, testosterone, FSH, $E_2$, prolactin (PRL), and corticosterone levels; 4) reproductive organ (i.e., testis and ventral prostate) weights; and 5) male mating behavior in Noble (NB/Cr) and Wistar-Furth (WF/NCr) rat strains to determine the genetic influence on the measured parameters. Maximal phenotypic divergence in male SDN-POA volumes was seen between NB/Cr versus WF/NCr and BDIX/Cr rats (among nine rat strains initially examined), with the average SDN-POA volume of NB/Cr male rats being significantly greater (≈30%) than that of either WF/NCr or BDIX/Cr males. Subsequent experiments investigated WF/NCr versus NB/Cr male rats in further detail. Significantly higher MBH-POA aromatase activity was seen in adult WF/NCr versus NB/Cr males, while MBH-POA 5α-reductase rates were not significantly different (within or between sex) for the two rat strains assayed. Serum LH levels were significantly higher (by greater than sixfold) in WF/NCr versus NB/Cr males, whereas testis organ:body weight and ventral prostate:body weight ratios in WF/NCr versus NB/Cr males were significantly smaller (by ≈6-fold for testis and ≈1.5-fold for prostate values). Serum FSH levels were significantly higher (by twofold) in WF/NCr versus NB/Cr males. However, serum testosterone levels were not significantly different, whereas $E_2$ levels were approximately twofold higher (but not significantly different) in WF/NCr versus NB/Cr animals. No significant differences were found in basal (i.e., nonstress) serum PRL or corticosterone levels between the WF/NCr and NB/Cr males. In male copulatory tests, NB/Cr males exhibited significantly more aggressive sexual behavior (e.g., in mounting, intromission, and ejaculation parameters) compared with WF/NCr males. Taken together, these findings indicate that WF/NCr males are, in general, low responders, whereas NB/Cr males are high responders to hormonal signals. The obtained data suggest that the correlative, phenotypic variation in SDN-POA volume (i.e., structure) and reproductive hormone patterns and mating behavior (i.e., function) of WF/NCr versus NB/Cr males is regulated by potentially $E_2$-mediated mechanisms that are genetically controlled.**

*corticosterone, estradiol, estradiol receptor, FSH, hormone action, hypothalamus, LH, male sexual function, prolactin, reproductive behavior, testes*

## INTRODUCTION

The importance of sex steroids influencing the sexual differentiation of the brain during specific periods of embryonic and neonatal development in mammals is well established [1–4]. However, interest in the genetic control of sexual differentiation has arisen during recent years due to the advancements in our understanding of the modulation of sex steroid hormone action that mediates brain development, gender identity, and sexual behavior [5–9]. Because several factors contribute to the genetic regulation of initiating/modulating sexually dimorphic neural development and function, we examined candidate neuroanatomical, hormonal, biological, and reproductive behavior parameters in the present study using many different inbred rat strains that are commercially available.

The medial basal hypothalamic (MBH) and preoptic areas (POA) are important neuroanatomical structures involved in the metabolism of steroid hormones, the modulation of neuroendocrine functions, and the regulation of sexual behavior [3, 10–13]. The two major enzymes involved in androgen metabolism, aromatase cytochrome P450 ($P450_{arom}$) and 5α-reductase, have been detected in many brain regions, including the MBH-POA [3, 10, 11, 14]. Additionally, both enzymes influence neuroendocrine systems, sexual behavior, and neural development [10–14]. In particular, the conversion of testosterone (T) to 17β-estradiol ($E_2$) by $P450_{arom}$ regulates the formation of sexually dimorphic neural structures in rodents during perinatal development [3, 10–12].

Notably, the concept of localized neural $E_2$ formation is exemplified by one of the most studied regions of the rodent brain, the sexually dimorphic nucleus (SDN) of the POA [3, 4, 10, 11, 15, 16]. In general, males exhibit significantly greater SDN-POA volumes than females [3, 4, 10, 11, 15, 16]. This sex difference is due to the availability of higher substrate concentrations of T in males versus females, the presence of greater $P450_{arom}$ activity that converts T to $E_2$ in males versus females, and local elicitation of estrogen receptor (ER)-mediated neuronal responses [3, 4, 10, 11, 15–18]. The precise function of the SDN-POA is somewhat controversial. However, LHRH cell bodies are located in this area, and evidence suggests that the SDN-POA regulates sexual behavior and gonadotropin levels in rodents [10, 11, 16, 19–26]. In fact, data from our labora-

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tory indicate a direct correspondence between SDN-POA volumes and sexual performance in adult male rats, which underscores the association between brain structure and mating behavior function [20, 26].

Recently, we demonstrated that phenotypic variation in $E_2$-regulated uterine responses is controlled by multiple, interacting quantitative trait loci (QTL) [27, 28]. Our results showed that allelic variants exist in the molecules or regulatory elements within the pathways leading to $E_2$-regulated responses [27, 28]. Given this finding and the role of $E_2$ in establishing neuroendocrine structure-function relationships, we undertook the present study to find out whether genetically controlled phenotypic variation exists in MBH-POA neuroanatomical structures and their associated functions.

## MATERIALS AND METHODS

### Animals

Inbred BDIX/Cr, BUF/NCr, COP/Cr, F344/NCr, Lew/NCr, LOU/MNCr, M520/NCr, NB/Cr, and WF/NCr male and female rats (35 days old) were obtained through the Animal Production Program of the National Cancer Institute (NCI; Frederick, MD). (A detailed history and description of the inbred strains of rat used in this study, with the exception of NB/Cr, can be obtained a http://rgd.mcw.edu/strain/. For NB/Cr, this information can be found in Technical Bulletin 1 at http://criver.com/techdocs/index.html#rats.). All animals were housed in a controlled environment under a reverse-lighting schedule (lights-off from 0600–1600 h) with a temperature from 23 to 25°C and free access to tap water and rat laboratory chow (Teklad Rat Diet; Harlan, Madison, WI). The animals remained in their colony home cages (four animals per cage) until brain histology was performed (≈45–48 days of age). In three separate experiments, brain and reproductive organ tissues were collected and serum hormones (i.e., gonadotropin and sex steroids) assayed (60–65 days of age; one animal per cage), male mating behavior tests (77–101 days of age; one animal per cage) conducted, and plasma prolactin (PRL) and corticosterone levels determined (130 days of age; one animal per cage). The animals and methods used in this study were approved by the Institutional Animal Care and Use Committee of Brigham Young University.

### Morphometric Analysis of the SDN-POA

Animals used to study the SDN-POA remained in their home cages for approximately 10–13 days after they were received from NCI. Male and female rats (≈45–48 days of age) were anesthetized with ketamine and acepromazine (4:1, v:v) at 0.15 ml per 100 g body weight. The animals were killed by intracardiac perfusion with 0.9% saline and then 10% formalin. Subsequently, the whole brain was immediately removed from the skull and stored in 10% formalin for at least 14 days before processing [29, 30]. The brains were frozen-sectioned at 50 μm in the De Groot plane. The sections were mounted onto slides and stained with thionin. Three independent observers traced the cross-sectional boundaries of the SDN-POA on the right side of each brain sample in successive sections using an overhead projecting system for tissue slides (McBain Instruments, Los Angeles, CA). Cross-sectional areas were calculated using a digitizing computer pad (Bioquant II; R & M Biometrics, Nashville, TN) that incorporated a correction for magnification. The volume of the SDN-POA was averaged and calculated by summing the serial cross-sectional areas and multiplying by the section thickness. All measurements were performed without knowledge of the sex or strain. A very high correspondence was found among the observers in determination of the SDN-POA volumes ($r = 0.92$).

### Cytochrome $P450_{arom}$ and 5α-Reductase Enzyme Activity Assays

In a separate group of animals, brain $P450_{arom}$ and 5α-reductase activities were determined in 60–65-day-old, age-matched male and female WF/NCr and NB/Cr rats [10, 13]. Also, because the in situ conversion of T to estrogens via the aromatase enzyme in brain during perinatal development plays an important role in determining SDN-POA characteristics, MBH-POA aromatase activity levels were determined in fetal male WF/NCr and NB/Cr animals (at Day 20 of gestation). The sex of each fetal animal was confirmed by the presence or absence of the testis in the pelvic cavity using a dissecting microscope. In either study, the animals were killed by decapitation, and trunk blood was collected. Brains were removed from the skulls, and the MBH-POA was isolated using landmark boundaries as described previously [31, 32]. The MBH-POA tissue samples were incubated in the presence of a saturating concentration of 1α,1β-$^3$H-T (3.0 μM) in a total volume of 0.2 ml of Dulbecco's modified Eagle's medium at pH 7.0–7.2. The reaction tubes were incubated for 1 h at 37°C. In adult animals only, MBH-POA $P450_{arom}$ and 5α-reductase activities were determined simultaneously. The $P450_{arom}$ activity was determined by isolating and quantifying the $^3H_20$ formed after release of the 1β-$^3$H from the labeled substrate. In contrast, the 1α-$^3$H of the labeled substrate remains with the 5α-reduced metabolites generated during the incubation. Subsequently, 5α-reductase activity was determined by thin-layer chromatography with cold steroid standards and quantified using scintillation counting. The methods for both assays have been validated and described in detail elsewhere [31–34].

The protein content for each MBH-POA tissue fragment was determined according to the method described by Lowry et al. [35]. Additionally, the protein content of each tissue fragment was standardized (i.e., the individual sample activity rates were divided by the mean protein content with respect to sex and strain) to ensure an accurate measurement of the assayed brain enzyme activities. However, the activity profiles did not change, even when the data were expressed as a function of the MBH-POA tissue fragments, thus indicating the accuracy with which the MBH-POA tissue samples were collected. The $P450_{arom}$ activity was expressed as fmol/h/mg protein for adult animals and pmol/h/mg protein for fetal data, whereas 5α-reductase activity was expressed in pmol/h/mg protein [31–34].

### LH, FSH, T, $E_2$, PRL, and Corticosterone RIAs

All blood samples were collected between 1000–1200 h, except those for the determination of PRL and corticosterone, which were collected at 1600–1700 h. From the obtained trunk blood, serum was prepared by centrifugation and stored at −20°C until assayed. Serum LH, FSH, T, and $E_2$ levels were determined in 60- to 65-day-old male rats. Under nonstress conditions (i.e., animals taken from their home cages and killed by decapitation within 10–15 sec), serum PRL and corticosterone levels were measured in 130-day-old males.

The LH and FSH levels were measured using validated

RIAs [36]. The reagents were obtained from the National Hormone and Pituitary Program, Bethesda, MD. The obtained results were expressed in terms of the LH-RP3 or FSH-RP2 reference standards, and the sensitivities of the assays were 0.07 ng/ml for LH and 0.4 ng/ml for FSH. The blood samples were run in single assays for either LH or FSH in duplicate, with intra-assay coefficients of variations less than 7% and 10%, respectively.

Levels of the sex steroid hormones, T and $E_2$ (using an ultrasensitive estradiol kit), and PRL were determined using a coated-tube RIA validated for rat serum (Diagnostic System Laboratories, Webster, TX). The sensitivities of the assays for T, $E_2$, and PRL were 0.1 ng/ml, 0.6 pg/ml, and 0.1 ng/ml, respectively. The samples were run in a single assay (in triplicate for T and in duplicate for $E_2$ and PRL) with high and low internal controls. The intra-assay coefficients of variations were less than 3% for T, 5% for $E_2$, and 4% for PRL.

Serum corticosterone levels were determined using a coated-tube RIA validated for rat serum (Diagnostic Products, Los Angeles, CA). The sensitivity of the corticosterone assay was 6 ng/ml, or 0.6 μg/dl. The samples were run in a single assay in duplicate with high and low controls. The intra-assay coefficient of variation was less than 6%.

### *Male Copulatory Behavior Analysis*

The sexual behavior tests were run in two trials [26, 30]. The male rats were 77–84 days old in the first trial and 87–101 days old in the second. The NB/Cr and WF/NCr male rats were tested during the dark cycle for spontaneous copulatory behavior with ovariectomized Sprague-Dawley female rats made receptive by hormonal injection (0.1 mg of estradiol benzoate followed 42 h later by 1 mg of progesterone s.c.). Plexiglas mating arenas (45 × 27 × 40 cm) were illuminated with one 25-W red light bulb. The female rats were introduced into the mating arena approximately 5 h after the progesterone treatment. In independent mating tests, the male rats were placed in the mating arena for 5 min before a receptive target female rat was introduced. Evaluation of male mating performance was initiated with the introduction of the receptive female and continued until the test male rat displayed mounting behavior following the first ejaculation. If no mounts were observed within the first 15 min of the mating session, then the first female rat was replaced with another receptive female. If the male rat did not mount the female rat within 30 min, the trial was terminated. If the male did not ejaculate but mounted the female rat within 30 min, then the session was extended until the male ejaculated or a 60-min interval expired.

All male rats were tested a second time approximately 7 days after the first test. In addition, the number of mounts and intromissions (i.e., mount and intromission frequency) to first ejaculation was quantified. The times to first mount, first ejaculation, and the time to first mount after the first ejaculation (or ejaculatory latency period) were recorded. Moreover, the total number of NB/Cr and WF/NCr animals ejaculating during the mating sessions was recorded.

In a separate experiment, 100-day-old male WF/NCr and NB/Cr animals (n = 10 per group) were studied in long-term mating tests (for 15 days). Random cycling WF/NCr or NB/Cr female rats (one at a time) were placed with one male rat (WF/NCr or NB/Cr) in their home cages, and the animals were observed for 15 min. Thereafter, the appearance of ejaculatory plug(s) under each cage was checked each day for 15 days. During the 15-day interval, each male rat had the opportunity to mate with three different (either of the same or a different strain) female targets.

### *Statistical Analysis*

For each biological and behavioral parameter quantified, the results were analyzed by one-way or two-way analysis (sex × strain) of variance (when appropriate), followed by Tukey's pair-wise comparisons to detect significant differences between groups ($P < 0.05$). For mating behavior data analysis, repeated measures detected significant differences between the rat strains, followed by post-hoc pair-wise comparison tests via a Student's Newman-Keuls method ($P < 0.05$). The percentage of males that ejaculated during the sexual behavior tests was determined using proportional statistics [37].

## RESULTS

In the initial inbred strain analysis, differences were observed in the SDN-POA volumes between male and female rats for all nine inbred strains studied (data not shown). The SDN-POA volumes of males ranged from two- to sixfold greater than females, with an average female SDN-POA volume of $6.44 \pm 0.64 \times 10^{-3}$ mm$^3$ and an average male volume of $15.44 \pm 1.34 \times 10^{-3}$ mm$^3$ ($P < 0.05$). Evidence for potentially significant differences in SDN-POA volumes was not seen in the females ($F = 0.41$, $P = 0.86$). In contrast, evidence for potentially significant differences was seen among the males ($F = 30.65$, $P < 0.05$), with NB/Cr males being significantly different from all other males ($P < 0.05$).

To verify the results observed with NB/Cr male rats in the initial strain distribution study, a larger cohort of male and female animals was studied. Additionally, two of the eight strains not exhibiting evidence for potentially divergent male SDN-POA volumes (i.e., WF/NCr and BDIX/Cr) were selected for further analysis. The SDN-POA volumes of NB/Cr males were approximately threefold larger than those of NB/Cr females, whereas in WF/NCr and BDIX/Cr males, the SDN-POA volumes were approximately twofold greater than in females (Fig. 1). No significant differences in SDN-POA volumes were seen between WF/NCr, BDIX/Cr, and NB/Cr females. The SDN-POA volume in NB/Cr males was approximately 30% greater ($P < 0.05$) than that of both WF/NCr and BDIX/Cr males as determined by morphometric coronal brain section measurements (Fig. 1). The male phenotypic differences in SDN-POA volumes among the three strains were even more dramatic if this parameter was expressed as a function of brain weight (i.e., NB/Cr SDN-POA volumes were ≈35% greater than WF/NCr or BDIX/Cr values; data not shown). The NB/Cr and WF/NCr rats were then selected as prototype inbred strains exhibiting maximally divergent male SDN-POA volumes and, therefore, were used in all subsequent studies.

The MBH-POA $P450_{arom}$ and 5α-reductase enzyme activities were determined in adult male and female NB/Cr and WF/NCr rats, or the MBH-POA $P450_{arom}$ enzyme activity was determined in prenatal NB/Cr and WF/NCr male animals. As seen above, in a similar fashion of significantly larger postnatal male versus female SDN-POA volumes, $P450_{arom}$ enzyme activity levels were significantly higher in male WF/NCr and NB/Cr rats as compared to female WF/NCr and NB/Cr rats (Fig. 2). However, WF/NCr males displayed significantly higher MBH-POA $P450_{arom}$ activity than NB/Cr males (Fig. 2).

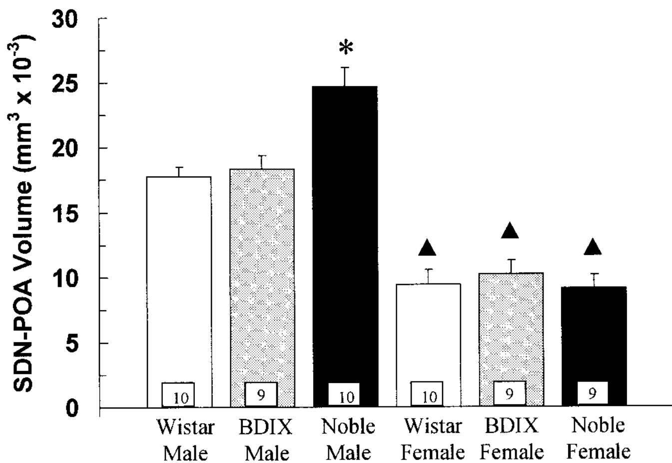


FIG. 1. The SDN-POA volumes in 45- to 48-day-old male and female Wistar (WF/NCr), BDIX/Cr, and Noble (NB/Cr) rats. Wistar, BDIX/Cr, and Noble male SDN-POA volumes were significantly larger ($P \leq 0.05$) than the corresponding female volumes (▲). The SDN-POA volumes of Wistar, BDIX/Cr, and Noble females were not significantly different ($P \geq 0.05$) from one another. However, the SDN-POA volumes of Noble males (*) were significantly greater (≈30%) than that of Wistar and BDIX/Cr males ($P \leq 0.05$). The number of animals analyzed in each group is shown at the base of each bar.

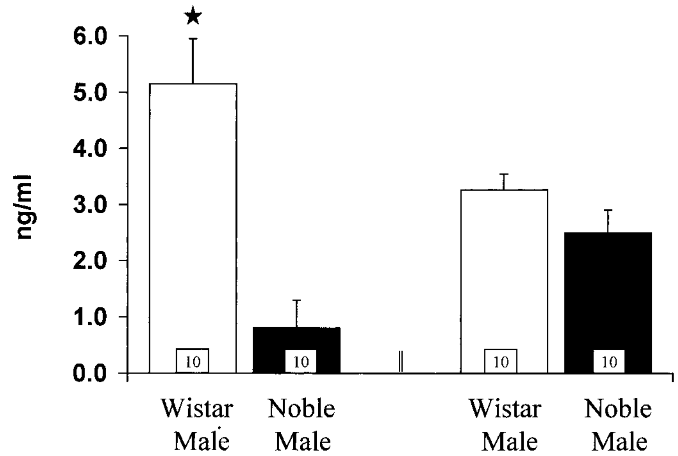


FIG. 3. Serum LH and T concentrations in age-matched (60- to 65-day-old), adult Wistar (WF/NCr) and Noble (NB/Cr) male rats. The LH concentration in Wistar animals (★) was significantly higher (by approximately sixfold) than in Noble rats ($P \leq 0.05$). No significant difference ($P \geq 0.05$) in plasma T levels was detected between Wistar versus Nobel rats. The number of animals tested is shown at the base of each bar.

When prenatal MBH-POA aromatase levels were examined, no significant differences were recorded between the WF/NCr versus NB/Cr males. At Day 20 of gestation, NB/Cr MBH-POA aromatase = 1.46 ± 0.1 pmol/h/mg protein (n = 10) versus WF/NCr MBH-POA aromatase = 1.50 ± 0.1 pmol/h/mg protein (n = 10; data not shown).

In contrast, no consistent significant strain differences or sexual dimorphisms were observed between adult male and female rats for MBH-POA 5α-reductase activity (data not shown). This result is not unexpected, because sex differences in 5α-reductase activity have not been reported previously [13, 14].

The LH levels in adult WF/NCr male rats were significantly higher (>6-fold) compared with NB/Cr males (Fig. 3). Therefore, circulating T levels were determined to assess the testes response to LH. No significant difference in T levels was seen between the two strains in adult animals (Fig. 3). However, when prenatal T levels were examined at Day 20 of gestation, NB/Cr males display significantly higher T levels than WF/NCr animals: NB/Cr = 1.1 ± 0.2 ng/ml (n = 10) versus 0.6 ± 0.1 ng/ml (n = 10) for WF/NCr values.

When FSH and $E_2$ levels were determined, circulating

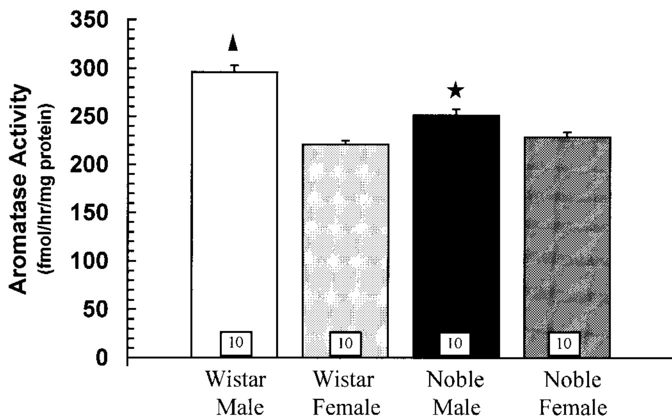


FIG. 2. Aromatase activity in the MBH-POA area of 60- to 65-day-old male and female Wistar (WF/NCr) and Noble (NB/Cr) rats. Significantly higher ($P \leq 0.05$) MBH-POA $P450_{arom}$ activity was seen in Wistar and Noble males compared with Wistar and Noble females. Significantly higher ($P \leq 0.05$) MBH-POA $P450_{arom}$ activity was seen in Wistar males (▲) compared with Noble males, whereas no significant difference ($P \geq 0.05$) in activity was detected between Wistar and Noble females. The number of animals assayed per group is shown at the base of each bar. ★, Significantly greater Noble MBH-POA aromatase levels compared to Wistar or Noble female values.

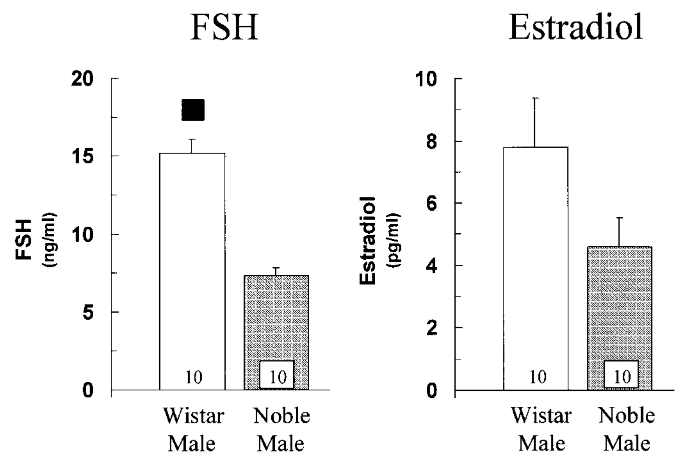


FIG. 4. Serum FSH and $E_2$ levels in age-matched (60- to 65-day-old), adult Wistar (WF/NCr) and Noble (NB/Cr) male rats. The FSH levels were significantly higher (by approximately twofold) in Wistar (■) versus Noble male animals ($P \leq 0.05$). Whereas $E_2$ levels were approximately 2-fold higher in Wistar vs. Noble males, this difference did not reach statistical significance ($P < 0.09$). The number of animals tested is shown at the base of each bar.

TABLE 1. Reproductive organ weight : body weight ratios in WF/NCr and NB/Cr males.*

| Variable | WF/NCr (n = 10) | NB/Cr (n = 10) |
|---|---|---|
| Body weight | 273.7 ± 3.4 | 302.5 ± 8.2† |
| Testis weight | 0.498 ± 0.063 | 3.225 ± 0.074† |
| Testis weight: 100 g body weight | 0.182 ± 0.024 | 1.069 ± 0.021† |
| Ventral prostate weight | 0.197 ± 0.007 | 0.323 ± 0.017† |
| Ventral prostate weight: 100 g body weight. | 0.072 ± 0.002 | 0.107 ± 0.005† |

* Body, testes, and prostate weights were determined in 60–65-day-old, inbred WF/NCr and NB/Cr males. All values are expressed as wet weight (g) ± SEM.
† $P \leq 0.05$; NB/Cr values significantly greater than Wistar values.

FSH levels in the adult WF/NCr male rats were significantly higher (by twofold) compared to NB/Cr males (Fig. 4). The serum $E_2$ levels were similar to the FSH pattern between the rat strains, in which WF/NCr males displayed approximately twofold greater values than the NB/Cr males; however, this difference was not significant ($P < 0.09$; Fig. 4).

No significant differences were observed in basal plasma corticosterone levels (mean ± SEM; WF/NCr = 22.7 ± 2.8 μg/dl vs. NB/Cr = 27.3 ± 2.3 μg/dl) or in basal PRL levels (WF/NCr = 1.5 ± 0.01 ng/ml vs. NB/Cr = 1.6 ± 0.06 ng/ml) males. (The PRL and corticosterone data are not shown graphically.)

The relationship between T levels and both testes weights and ventral prostate weights was examined in age-matched, 60- to 65-day-old rats (Table 1). Testes weights were significantly less (by sixfold) in WF/NCr compared to NB/Cr animals. Similarly, ventral prostate weights were significantly less in WF/NCr males. When the data were expressed as a function of body weight, the results were similar, in that WF/NCr males exhibit significantly lower values compared with NB/Cr males for both parameters (Table 1).

Sexually naïve WF/NCr and NB/Cr male rats were evaluated for a variety of male copulatory parameters. In an extraordinary fashion, NB/Cr males mounted receptive females fivefold more quickly (<40 sec) than did WF/NCr males (200 sec; Fig. 5). For NB/Cr animals, the time to ejaculation was significantly less compared to the WF/NCr group (610 vs. 1360 sec; Fig. 5). Furthermore, the number of mounts and intromissions leading to the first ejaculation were significantly fewer in NB/Cr versus WF/NCr males (data not shown). During the two mating behavior sessions, 100% (12/12) of the NB/Cr males ejaculated, whereas only 42% (5/12) of the WF/NCr males ejaculated (Fig. 5). Finally, the ejaculatory latency period of NB/Cr males was significantly shorter (310 sec) than that of WF/NCr males (425 sec; Fig. 5).

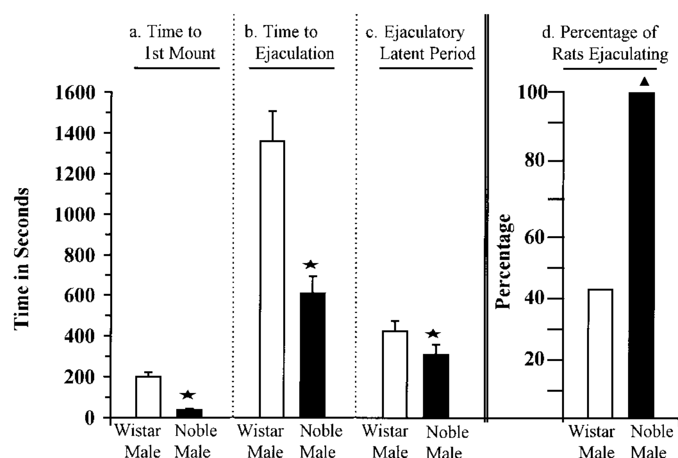


FIG. 5. Sexual behavior in age-matched, adult Wistar (WF/NCr) and Noble (NB/Cr) males. In all parameters examined, Noble males (★) exhibited significantly greater sexual aggressiveness ($P \leq 0.05$) and percentage of Noble rats ejaculating (▲) compared with Wistar males. Twelve males of each inbred strain were studied. Only sexually active animals were included in the data presented in **a–c**. **a**) Time to first mount (i.e., mount latency). **b**) Time to ejaculation. **c**) Ejaculatory latency period (in sec). **d**) Percentage of Wistar and Noble rats ejaculating in two mating tests.

Finally, in long-term mating studies in which adult, randomly cycling WF/NCr or NB/Cr female rats were placed with adult WF/NCr or NB/Cr males, respectively, NB/Cr males displayed very aggressive mating behaviors. Notably, NB/Cr males mounted females even when they did not display lordosis (i.e., females that presumably were not in estrus). In some instances, the aggressive mating behavior of some NB/Cr males was so intense that NB/Cr females not in estrus had to be taken out of the male's cage due to injury caused by the NB/Cr males. In contrast, the WF/NCr males appeared to be more timid than NB/Cr males in their expression of mating behavior. However, if WF/NCr and NB/Cr males were allowed to mate with WF/NCr or NB/Cr females, respectively, for a 15-day interval, then the evidence of mating between WF/NCr and NB/Cr males was similar (9 of 10 WF/NCr males ejaculated, whereas 10 of 10 NB/Cr males ejaculated).

## DISCUSSION

Sex steroids play an important role in the sexual differentiation of the brain during specific periods of embryonic and neonatal development in mammals [1–4]. Recently, however, interest in the genetic control of sexual differentiation has surfaced in light of abundant data supporting the dominant theory of differentiation by the action of sex steroid hormones mediating brain development, gender identity, and sexual behavior [3–9]. We have shown recently that $E_2$-regulated responses in the uterus are controlled by QTL [27, 28]. This observation suggests that phenotypic variation observed in the developmental, physiological, and endocrinological effects of $E_2$ in other organs may also be due to QTL. Given this finding and the role of $E_2$ in establishing neuroendocrine structure-function relationships, we evaluated SDN-POA volumes in nine different inbred strains of rats.

Significant differences in SDN-POA volumes were observed in NB/Cr rats from both WF/NCr and BDIX/Cr males. Therefore, male sexual behavior (prenatal and adult) MBH-POA $P450_{arom}$ and $5\alpha$-reductase activities; serum

LH, FSH, T, $E_2$, PRL, and corticosterone levels; and reproductive organ weights were examined in NB/Cr and WF/NCr rats. Our results establish that a genetically controlled, phenotypic variation exists in SDN-POA volumes and sexual behavior between the two inbred strains.

The strikingly positive correlation between SDN-POA volumes and copulatory aggressiveness further supports the connection between this important brain structure and sexual behavior [4, 15, 16, 20–26]. The NB/Cr male rats displayed significantly more aggressive sexual behavior, which correlates with their larger SDN-POA volumes. In contrast, WF/NCr males were less sexually aggressive and exhibited correspondingly smaller SDN-POA volumes. Because stress hormones can inhibit reproductive function and influence prostate characteristics in rats [38, 39], plasma PRL and corticosterone levels were assayed, but these were not significantly different between the two rat strains.

The SDN-POA volumes are established during perinatal development by 1) the presence of $P450_{arom}$ in the MBH-POA region, 2) the availability of T for conversion to $E_2$, and 3) the elicitation of local $E_2$-regulated neuronal responses via ER [3, 10–12, 15, 16]. Additionally, during perinatal development, other important factors, such as calcium-binding proteins that protect neurons from cell death, along with circulating levels of progesterone and progestin receptors may be involved in contributing to the formation of sexually dimorphic nuclei [40–44].

Regarding the establishment of SDN-POA volumes and the finding that NB/Cr male rats displayed significantly larger SDN-POA volumes than WF/NCr males postnatally, we determined prenatal MBH-POA aromatase levels in these animals. No significant differences in MBH-POA aromatase were recorded in fetal WF/NCr versus NB/Cr male animals, but significantly higher levels of T were seen in NB/Cr versus WF/NCr males at Day 20 of gestation. This may suggest higher substrate (i.e., T) availability to the brain during an interval when aromatase activity levels are highest in prenatal development [10, 11].

Also, it is not known whether, during perinatal development, alterations occur in the transmission of $E_2$ signals via divergent ER-$\alpha$ and/or -$\beta$ systems expressed in certain brain structures [45, 46] and the various forms of the ER through which estrogen hormonal action is transmitted [10, 47, 48]. Recent studies examining ER-$\alpha$ knockout mice provide insight regarding the importance of estrogen-mediated events playing a regulatory role in sexual behavior [49, 50] that may be operational in the divergent behavioral patterns observed in the WF/NCr versus NB/Cr animals in the present study.

It is not immediately apparent why significantly higher adult levels of MBH-POA $P450_{arom}$ activity in WF/NCr male rats and the putative local production of $E_2$ is accompanied by significantly less aggressive sexual behavior [22, 24, 51–53]. Based on known mechanisms, one would predict that higher local concentrations of $E_2$ produced within the basal hypothalamic region, by adequate circulating levels of T via its conversion by the aromatase enzyme, would stimulate greater sexual behavior in WF/NCr males. Implantation of $E_2$ into the MBH-POA of castrated rats activates masculine sexual behavior, whereas blocking brain $P450_{arom}$ activity inhibits male sexual behavior [10, 52, 53]. The present results are in agreement with those of a previous study, in which significantly greater MBH-POA $P450_{arom}$ activity was seen in control, Wistar intact males compared to Sprague-Dawley rats [54]. In reference to the sexual behavior patterns observed in NB/Cr males, one can infer that the steroid-receptor systems are functional, because a robust display of mating behavior was observed. This interpretation is also supported by the observation that NB/Cr rats develop spontaneous, hormone-dependent tumors in reproductive tissues [55–57]. Finally, our laboratory has reported a direct correspondence between SDN-POA volumes and sexual performance in adult male rats that underscores the association between brain structure and mating behavior function [20, 26].

It is intriguing to consider that the phenotypic variation observed in WF/NCr versus NB/Cr animals may be due to $E_2$-regulated neuroanatomical and sexual behavior responses that, in turn, are controlled by QTL. Under this hypothesis, NB/Cr rats are considered to be quantitatively ''high'' responders to $E_2$, thereby leading to greater SDN-POA volumes, increased aggressiveness in male sexual behavior, and lower LHRH-LH secretion patterns. In contrast, WF/NCr are ''low'' responders to $E_2$ and, therefore, have reduced SDN-POA volumes and are not as sexually aggressive. The mechanisms underlying these divergent responses are unknown. However, our recent observations, along with those of other investigators, demonstrating that QTL control the phenotypic variation observed in uterine growth following $E_2$ treatment or estrogen-dependent pituitary tumor growth [28, 58] suggest that a similar mechanism may underlie the differences in SDN-POA volumes. In the high-responder NB/Cr rat, it is conceivable that $E_2$ elicits significantly more neuronal migration/survival via known $E_2$-mediated molecules, such as *bcl*-2, which inhibits apoptotic mechanisms during the perinatal developmental period when the SDN-POA is being established [59–61]. In WF/NCr rats, the same level of $E_2$ results in less neuronal migration/survival and smaller SDN-POA volumes. The molecular characterization of loci controlling such responses is of significance in understanding the mechanisms underlying the development of hypothalamic, sexually dimorphic nuclei as well as their structure-function relationships and phenotypic variation in sexual behavior.

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