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HORMONAL CONTROL OF A DEVELOPING NEUROMUSCULAR SYSTEM

I. Complete Demasculinization of the Male Rat Spinal Nucleus of the Bulbocavernosus Using the Anti-androgen Flutamide[1]

S. MARC BREEDLOVE[2] AND ARTHUR P. ARNOLD

Department of Psychology and Laboratory of Neuroendocrinology, Brain Research Institute, University of California, Los Angeles, California 90024

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Abstract

Prenatal treatment of male rats with the anti-androgen, flutamide (FL), demasculinizes the sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) by reducing the number of SNB neurons, the size of the somas and nuclei of SNB neurons, and the size of their target muscles in adulthood. However, FL does not affect mounting or the traditional, postural measure of intromission, indicating that the SNB system does not play a major role in the mediation of these particular behaviors. Postnatal testosterone propionate (TP) treatment of male rats castrated on the day of birth results in more male copulatory behaviors in adulthood and masculinizes all measures of the SNB system. The postnatal masculinization by TP is more pronounced in males treated prenatally with FL, for morphological but not behavioral measures. The combined treatment of prenatal FL and day 1 castration without TP therapy results in a male with a completely demasculinized SNB system. Specifically, such males have SNB neurons that are as scarce and as small as those of females and, like females, they lack the target muscles of the SNB. These results support the hypothesis that perinatal androgens normally direct the sexually dimorphic development of the SNB and its target muscles.

In the male rat, the bulbocavernosus (BC) and levator ani (LA) muscles are innervated by about 200 motoneurons which can be readily distinguished as a nucleus in the lumbar spinal cord. This spinal nucleus of the bulbocavernosus (SNB) is not discernible as a nucleus in female rats because the densely staining neurons in this region are only about one-third as numerous and one-half as large as those in the male (Breedlove and Arnold, 1980, 1981). Similarly, the target muscles of the SNB are absent in normal adult females. The motoneurons of the adult male SNB accumulate radioactivity following injections of tritiated testosterone (T) or dihydrotestosterone (DHT), but not estradiol (E) (Breedlove and Arnold, 1979).

The sexually dimorphic appearance of the SNB and the hormone accumulation by SNB neurons in adulthood suggest that perinatal androgens may be responsible for the sexually dimorphic development of this system. This conjecture is confirmed by the markedly feminine appearance of the SNB in male testicular feminized (*tfm*) rat mutants which have a smaller number of androgen receptors (Naess et al., 1976; Fox, 1975). Further support for the role of androgens in SNB masculinization is found in the increased number of SNB neurons in adult female rats when they are treated perinatally with testosterone propionate (TP) or dihydrotestosterone propionate (DHTP) (Breedlove and Arnold, 1983), but not when treated neonatally with estradiol benzoate (EB) (Breedlove et al., 1982).

The present experiments were designed to address further the hypothesis that androgens guide the masculinization of the SNB, specifically by analyzing the effects of the nonsteroidal anti-androgen, flutamide (FL; Neri et al., 1972), on the SNB of male rats. Prenatally administered FL has been reported to cause deficits in the copulatory behavior of male rats (Clemens et al., 1978). There were three principal reasons for attempting to

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[2] To whom correspondence should be addressed at his present address: Department of Psychology, University of California, Berkeley, CA 94720.

manipulate the appearance of the SNB with FL: (*1*) FL demasculinization of the SNB would provide evidence that estrogen receptors do not play a role in the masculinization of this nucleus, since FL is not known to interfere with estrogen receptors; (*2*) FL demasculinization of the SNB system would also indicate that androgen stimulation is necessary for the masculinization of the SNB, even in genetically normal males; and (*3*) the ability to demasculinize the SNB with a treatment such as FL would provide a valuable research tool complementing the ability to masculinize the SNB with androgens.

The present results show that when given prenatally, FL very successfully prevents the masculinization of the SNB and its target muscles but does not interfere with the conventional measures of sexual behavior in male rats. From these results we infer that androgens, but neither estrogens nor any nonhormonally mediated genetic mechanisms, are crucial for the normal male development of the SNB system.

Materials and Methods

After female rats displayed at least two regular 4-day cycles, they were left in a group cage with males on the day of proestrus. If sperm remnants were seen in the vaginal smear the following day, this day was designated as day 1 of gestation. From day 1 until day 10 of gestation, pregnant dams were gently handled each day. Rat fetuses were treated prenatally with flutamide by injecting the pregnant dam daily with 5 mg of flutamide in 0.1 ml of 1,2-propanediol (propylene glycol; PG), subcutaneously. This treatment was given from day 11 of gestation up to and including the day before birth. The dams of control pups were injected with PG vehicle only. All dams delivered normally on day 23 of gestation (day 1 postnatal). On the day of birth the ano-genital distance (AGD) of each pup was measured. In addition, all pups were castrated and identified individually by means of toe clipping. Because prenatal FL treatment did not alter the appearance of the gonads at birth, day 1 castration provided the opportunity to determine the sex of the animals. This method of sex determination was necessary because prenatal FL greatly reduced the AGD of males, making male and female AGDs indistinguishable. Some female pups were sacrificed, leaving each dam with eight pups.

It is possible that dams treated with flutamide might release the anti-androgen in their milk supply following delivery. This potential contamination could obscure independent manipulation of pre- and postnatal hormonal factors. In order to detect such a continued action of flutamide postnatally, one-half of the animals from each group were cross-fostered. For example, one-half of the pups exposed to flutamide prenatally were cross-fostered on the day of birth to a dam that had received propylene glycol (PG) vehicle and had that same day delivered a litter of prenatal PG pups, one-half of which were cross-fostered to a dam that had received flutamide. Beginning on the day of birth and on each alternate day until postnatal day 11, pups were injected with either 1 mg of testosterone propionate (TP) in 0.05 ml of sesame oil, s.c., or oil vehicle alone. When pups were injected, the needle puncture was sealed with flexible collodion. Thus the final experimental design consisted of eight groups of male pups in a simple three-way analysis of variance with the following factors: prenatal treatment (either flutamide or PG vehicle), postnatal treatment (either TP or oil vehicle), and fostering condition (either cross-fostered or left with the natural mother).

All pups were weaned on day 21 of life and housed in same-treatment groups of 3 to 4. In order to equalize the activational influence of androgens, all rats were given daily injections of TP (2 mg/kg, s.c.) in a sesame oil vehicle (5 mg of TP/ml) from day 68 of life until sacrifice on days 101 to 103. The masculine copulatory behavior of all animals was tested beginning 22 days after androgen treatment commenced. Each animal was tested in four sessions, with 2 days of rest between each. Test sessions were conducted during the dark portion of the light cycle, in the room where animals were raised and housed. Each session consisted of leaving the test animal alone in a glass aquarium (30 × 60 × 40 cm high) for 10 min, then introducing a hormonally primed, receptive stimulus female, which was replaced by another receptive female after 10 min. Ten minutes after the introduction of the second stimulus female, the session was ended. Masculine copulatory behavior of the test animals was measured by the postural correlates of mounting, intromission, and ejaculation. A mount was counted if it was accompanied by pelvic thrusting, but not intromission. An intromission was defined as a mount ending with a springing, backward dismount. Ejaculation was defined as a mount followed by a rearing and flexion of the forelimbs, usually accompanied by a high-pitched vocalization. In normal males these behaviors are quite unmistakable and have been correlated with literal intromission of the penis and ejaculation of semen. In addition to measuring the number of such behaviorally defined mounts, intromissions, and ejaculations, the latencies for each were measured. If an animal failed to show a particular behavior, a maximal latency of 1200 sec was assigned for that session. The ratio of the number of mounts with intromission compared with the total number of mounts was computed as "intromission efficiency." For those animals displaying ejaculation, the post-ejaculatory interval before the next intromission and the inter-ejaculatory interval were measured. The stimulus females were made responsive with subcutaneous injections of 20 μg of estradiol benzoate approximately 54 and 30 h prior to the test session, and a single injection of 0.5 mg of progesterone 6 h before the test.

Animals were sacrificed with sodium pentobarbital and were perfused intracardially with saline followed by buffered 10% formalin. The spinal cords from lumbar segment 4 to sacral segment 1 were removed and postfixed at least 1 week in buffered formalin. The perineal muscle complex from each animal, consisting of the muscles LA, BC, and ischiocavernosus and the base of the penis to which these muscles attach, was also removed and postfixed.

Spinal cords were frozen-sectioned at 50 μm and alternate sections were mounted and stained with thionin. A "blind" observer then counted the number of nuclei of densely staining cells in the region occupied by the SNB in normal males: 200 to 400 μm below the central canal, within 250 μm of the midline in lumbar segments 5 and

6. A dozen of these neurons were chosen at random and their somas were traced through a camera lucida. The mean area of these 12 cell somas provided a single score for each animal for analysis. This sampling procedure provides a quite sensitive measure of somatic and nuclear profile size, since analysis of variance detected highly significant effects of all three manipulations on somatic area (see Fig. 3, results below). The area of the nuclei provided an estimate of the mean nuclear diameter for use in Konigsmark's (1970) formula to correct for split nuclei error. This correction procedure is more fully described in Breedlove and Arnold (1981).

After several weeks of fixation, the perineal muscle complex from each animal was briefly dried with a paper towel to remove excess formalin, and then weighed. Additionally, a sample of the levator ani was removed. The number of LA fibers was taken to be representative of the total number of SNB target fibers, i.e., of both the LA and BC, because of the following considerations. The architecture of the BC fibers makes estimation of their numbers difficult. However, the presence or absence of the BC and LA muscles in individuals in different groups was perfectly correlated, and the estimate of LA fibers was correlated with the overall perineal muscle complex weight ($r = +0.6$, $p < 0.001$, $N = 31$ rats with muscles). The LA sample was stained overnight with 2% osmium tetroxide, dehydrated in alcohol, cleared in acetone, and then embedded in a soft epoxy resin plastic mixture. This mixture consisted of 110 parts dodecenylsuccinic anhydride, 65 parts Poly/Bed 812 (Polysciences, Inc., Warrington, PA), and 3 parts 2,4,6-tri-(dimethylaminomethyl)phenol. The plastic embedded muscles were left in a 58°C oven overnight to harden and then were sectioned 20 μm thick in the plane perpendicular to the fibers, using a sliding microtome. The sections were mounted on microscope slides using fresh resin mixture which was then hardened in an oven overnight. To estimate the number of fibers in the LA sample, the total area of the LA cross-section was divided by the mean area of 24 randomly sampled individual fibers. This estimate represents the number of LA fibers on one side and is valid as long as there is no branching of LA muscle fibers. We found no evidence of such branching since, for several animals, the total number of muscle fibers was counted from three locations in the longitudinal extent of the LA and were found to be quite comparable to estimates calculated as above. The number of muscle fibers appeared constant over the length of the muscle. Additionally, several LA muscles were cut longitudinally, but no evidence of fiber branching was seen.

Statistical analysis consisted of simple three-way analyses of variance (prenatal treatment × postnatal treatment × fostering condition). This analysis was applied to the following measures: number of SNB neurons, cross-sectional area of SNB somas, cross-sectional area of SNB neuronal nuclei, weight of perineal muscle complex, number of LA fibers on one side, and mean number of mounts, intromissions, and ejaculations. This analysis was also applied to the intromission efficiency and mean latencies for the three copulatory behaviors, following an angular transformation to approximate a normal distribution (Sokal and Rohlf, 1969, p. 386). For this purpose, latencies were converted to proportions of the maximum latency (1200 sec). Five rats from each of the eight groups were randomly chosen for morphological and behavioral analyses. Rats from at least three different litters made up the five subjects for each group.

Results

Number of SNB neurons. Prenatal flutamide treatment of male rats resulted in significantly fewer SNB neurons ($p < 0.001$) compared to PG-treated controls. Postnatal TP treatment resulted in significantly more SNB cells than did oil treatment ($p < 0.001$), although this postnatal treatment could not completely overcome the demasculinizing effects of prenatal flutamide exposure (Figs. 1 and 2). Because there was no significant effect of cross-fostering on SNB neuron number, Figure 2 represents the mean number of SNB cells in the groups combined across fostering conditions. There was a significant interaction between pre- and postnatal treatments ($p < 0.01$, Fig. 2), apparently because postnatal TP had a much greater effect on rats that had been treated prenatally with flutamide.

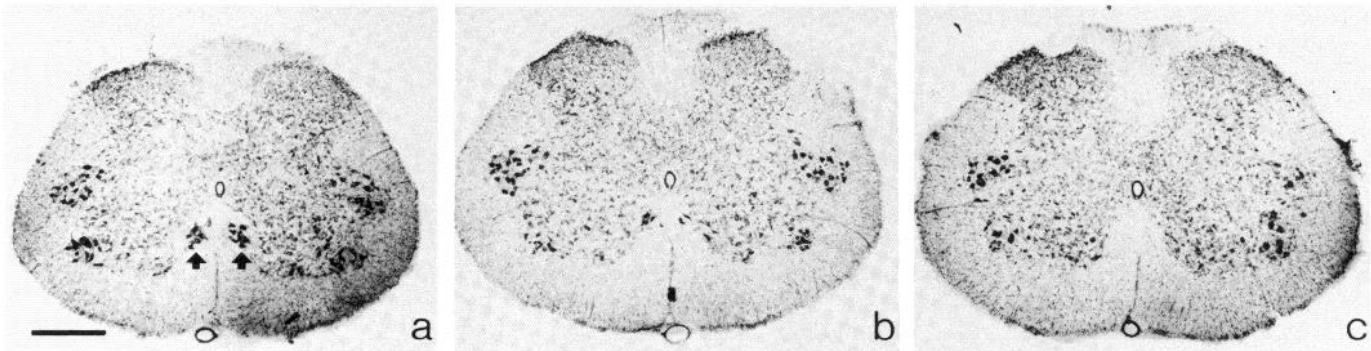

Figure 1. Photomicrographs of thionin-stained sections from the fifth lumbar segment of the adult rat spinal cord in the region of the spinal nucleus of the bulbocavernosus (SNB). *a*, Transverse section of the spinal cord of a male exposed to vehicle prenatally, castrated at birth, but given TP therapy just after birth. The *arrows* point to the SNB, which is morphologically identical to that of a normal male (e.g., see Breedlove and Arnold, 1983, Fig. 1*a*). *b*, Transverse section from a male given the antiandrogen flutamide prenatally, but given TP postnatally. Such males have fewer, smaller SNB cells and fewer target muscle fibers than do control males. *c*, Males given flutamide prenatally, castrated at birth, and given oil postnatally have spinal cords that are indistinguishable from those of normal females. Additionally, 9 of 10 such males, like normal females, totally lack the SNB target muscles. *Scale bar*, 500 μm; sections are 50 μm thick.

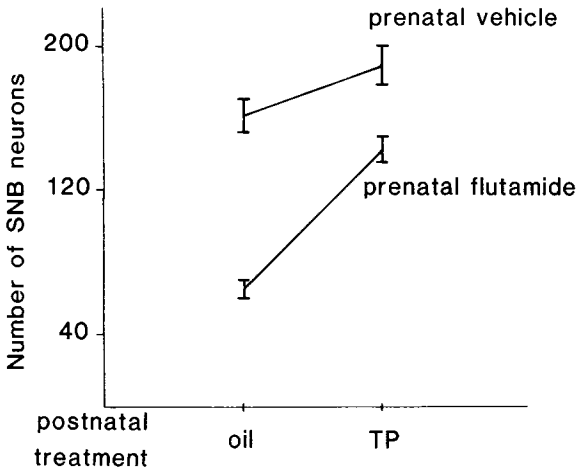

Figure 2. Prenatal treatment with the anti-androgen, flutamide, significantly demasculinizes the number of SNB motoneurons in male rats. Postnatal treatment with TP partially compensates for this demasculinization. All rats were castrated on the day of birth. Males treated prenatally with flutamide and given only oil postnatally have about as few SNB neurons (65.0 ± 4.8, *lower left* on graph above) as do control females (66.6 ± 4.5, not depicted above).

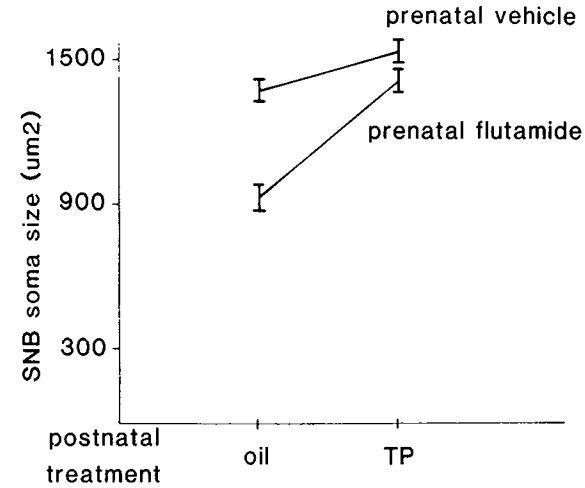

Figure 3. Prenatal treatment with the anti-androgen, flutamide, significantly demasculinizes the soma size of SNB motoneurons in male rats. Postnatal TP treatment partially compensates for this demasculinization. All rats were castrated on the day of birth and received TP treatment in adulthood prior to sacrifice.

SNB soma cross-sectional area. Prenatal flutamide resulted in smaller SNB soma areas ($p < 0.001$), while postnatal TP led to an increased SNB soma size ($p < 0.001$). Again, there was a significant interaction between pre- and postnatal treatments ($p < 0.001$), which is displayed in Figure 3. There was a significant main effect of cross-fostering on SNB soma size ($p < 0.01$), but there were no significant interactions of fostering conditions with any other factor. Cross-fostering resulted in larger SNB somas regardless of whether the pups were fostered by a dam that had received flutamide or PG vehicle. However, since there were no interactions of cross-fostering with any other factor, Figure 3 displays soma areas collapsed across fostering conditions.

SNB neuronal nuclei cross-sectional area. The size of the nuclei of SNB neurons was smaller following prenatal FL ($p < 0.001$) and larger after postnatal TP ($p < 0.001$), but there was no significant interaction of the pre- and postnatal treatments (Table I). There were no significant effects of cross-fostering.

Perineal muscle weight. Prenatal flutamide decreased perineal muscle complex weight ($p < 0.001$), and postnatal TP increased muscle weight ($p < 0.001$; Table II). TP had a significantly greater masculinizing effect on animals that had received prenatal flutamide rather than prenatal PG ($p < 0.001$). While cross-fostering did not have a significant main effect on muscle weight, there was a significant interaction of fostering and prenatal treatment ($p < 0.05$). Specifically, cross-fostering resulted in increased muscle weight of animals receiving prenatal PG ($p < 0.05$) but did not alter the muscle weight of animals treated with flutamide prenatally ($p > 0.05$; Table II).

Number of LA muscle fibers. The estimated number of LA muscle fibers was decreased by prenatal FL ($p < 0.001$) and increased by postnatal TP ($p < 0.001$; Fig. 4). Once again, postnatal TP had a significantly more masculinizing effect on animals treated with prenatal FL ($p < 0.01$). There were no significant effects of cross-fostering on the number of LA fibers.

TABLE I

The cross-sectional area of the nuclei of SNB neurons of adult male rats treated prenatally with the anti-androgen, flutamide, or PG vehicle

All pups were castrated on the day of birth. Postnatally, pups received either TP or oil vehicle. Standard errors of the mean are given, based on $N = 10$ animals per group.

	Postnatal Treatment	
	Oil	TP
	μm^2	
Prenatal flutamide	162.0 ± 9.89	215.1 ± 8.07
Prenatal PG vehicle	226.8 ± 7.04	251.0 ± 13.04

TABLE II

The mean weight (± standard error of the mean), in grams, of the perineal muscle complex from adult male rats castrated on the day of birth

Prenatal treatment with the anti-androgen, flutamide (FL), decreased the perineal muscle weight, compared to rats prenatally treated with propylene glycol (PG) vehicle. Postnatal TP increased muscle weight, especially in rats treated with FL prenatally. Half of the pups treated prenatally with flutamide were cross-fostered on the day of birth to dams that had received PG injections. Similarly, half of the pups treated prenatally with PG were cross-fostered to flutamide-treated dams. Other pups were left with their natural mothers. Cross-fostering significantly increased muscle weight only in animals prenatally treated with the PG vehicle. $N = 5$ animals per group.

	Postnatal Treatment			
	Oil		TP	
	Cross-fostering	Natural fostering	Cross-fostering	Natural fostering
Prenatal FL	0 (0)	0.10 (0.1)	1.07 (0.05)	1.03 (0.06)
Prenatal PG	1.20 (0.05)	1.03 (0.05)	1.78 (0.03)	1.59 (0.08)

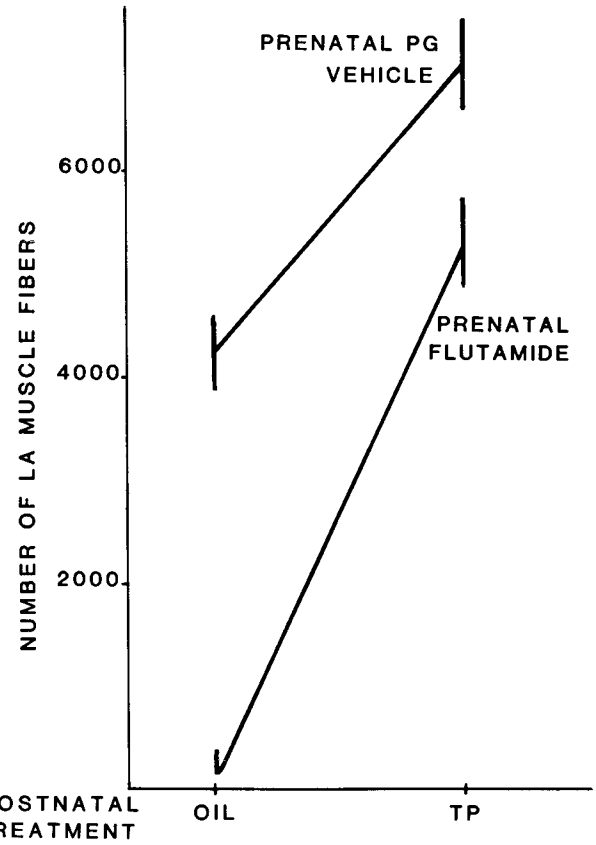

Figure 4. The number of fibers in the muscle levator ani (LA) is significantly reduced by prenatal treatment of male rats with the anti-androgen, flutamide. In fact, only 1 of the 10 males receiving prenatal flutamide and postnatal oil had the SNB target muscles in adulthood. Postnatal TP treatment increases the number of LA fibers. Postnatal TP treatment had a greater effect in animals receiving prenatal flutamide.

TABLE III

The mean number of intromissions displayed per 20-min test session by male rats treated prenatally with flutamide or vehicle

All pups were castrated at birth and treated with either TP or oil neonatally. The rats also received TP treatment in adulthood before and during testing. Analysis of variance revealed no effect of prenatal flutamide treatment or neonatal cross-fostering on intromission behavior. Neonatal TP treatment, however, significantly augmented intromission.

	Neonatal Treatment	
	Oil	TP
Prenatal flutamide	2.2 ± 0.58	5.7 ± 1.85
Prenatal vehicle (PG)	2.0 ± 0.63	4.8 ± 1.19

Masculine copulatory behavior. Neither the frequency nor latency of mounting behavior was significantly affected by either prenatal or postnatal androgen manipulation ($p > 0.05$, three-way analysis of variance, data not shown). Intromission frequency (Table III) and latency were altered by postnatal TP treatment ($p < 0.02$) but were not affected by prenatal FL exposure ($p > 0.05$). There were no significant interaction effects or effects of cross-fostering on either mounting or intromission. Four of the animals exhibited ejaculatory behavior once each. Three of these rats were treated with vehicle prenatally and TP postnatally, and were left with their biological mother. The fourth animal received flutamide prenatally and TP postnatally, and was cross-fostered at birth. Thus, while there was a significant three-way interaction of prenatal, postnatal, and fostering conditions on the number of ejaculations ($p < 0.02$), no single factor exhibited a predominant influence on ejaculation under the present testing conditions.

Fetus and pup survival. There was no appreciable mortality caused by either the prenatal or postnatal treatments. All FL- and PG-treated dams delivered live pups with a mean of 10.2 pups per FL litter and 12.3 pups per PG litter. Following live birth and day 1 manipulations, only 2 of 27 PG-treated pups and 3 of 32 FL-treated pups died before sacrifice on days 101 to 103 of life. On the day of birth, the AGD of males exposed to prenatal FL was 2.61 ± 0.07 (SEM, $N = 20$) mm, while that of males exposed to prenatal PG vehicle was 4.57 ± 0.06 ($N = 20$). The AGD on the day of birth of females exposed to prenatal FL was 2.43 ± 0.08 mm ($N = 14$), while that of those exposed to prenatal PG was 2.55 ± 0.04 mm ($N = 18$).

Discussion

The anti-androgen flutamide significantly demasculinized the SNB and its target muscles. Prenatal flutamide resulted in fewer SNB neurons, which had smaller somas, smaller neuronal nuclei, and fewer target muscle fibers. Postnatal TP treatment, on the other hand, resulted in significantly more SNB neurons which had larger somas, larger nuclei, and more target muscle fibers. The demasculinization of the SNB system by anti-androgen, the masculinizing effect of androgens in the present and other studies (Breedlove and Arnold, 1983; Breedlove et al., 1982), and the feminine appearance of the SNB in *tfm* rats (Breedlove and Arnold, 1981) leave little doubt that androgens are normally responsible for the sexually dimorphic development of the SNB system.

There was also significant interaction between the pre- and postnatal treatment effects on virtually every measure of the SNB system, including SNB cell number, SNB soma size, perineal muscle weight, and number of LA muscle fibers. The interaction consisted of a much more pronounced masculinizing effect of TP when given to flutamide-treated rather than vehicle-treated pups. This result implies that the SNB system of a rat receiving normal male levels of androgen prenatally is not very sensitive to postnatal androgen deprivation. Conversely, a rat receiving little prenatal androgen exposure, such as a normal female or a male deprived of androgen action by prenatal flutamide, *would* be sensitive to TP treatment immediately after birth. Thus, androgens present during either the prenatal or postnatal period can significantly compensate for a lack of androgens during the other period. This compensatory hypothesis is supported by two other findings. First, day 1 castration of male rats caused a statistically nonsignificant loss of SNB neurons, but treatment of female rats with DHTP just after birth caused a significant increase in the number of SNB neurons seen in adulthood (Breedlove and Arnold, 1983).

Previous studies have implied that the aromatized metabolite of testosterone, estradiol (E), is primarily responsible for masculinization of the brain (McEwen et al., 1977). For example, E alone is effective in masculinizing the pattern of gonadotropin release (Gorski and Wagner, 1965), lordosis behavior (Christensen and Gorski, 1978), and masculine copulatory behavior (Sodersten and Hansen, 1978). Furthermore, T is known to convert readily to E in the rat CNS (Naftolin et al., 1975). However, there is now a good deal of evidence that androgens *per se* guide the masculine development of the SNB without requiring aromatization. First, male *tfm* rats with normal levels of brain E receptors and subnormal levels of brain androgen receptors (Olsen and Fox, 1981) have a markedly feminine SNB (Breedlove and Arnold, 1981). Second, a single injection of TP (1 mg), but not EB (100 μg), on day 2 of life masculinized the number of SNB cells in adult females (Breedlove et al., 1982). Third, the non-aromatizable androgen, DHTP, when delivered immediately after birth, leads to a greater number and size of SNB motoneurons (Breedlove and Arnold, 1983). Finally, the present demonstration that prenatal flutamide demasculinizes the SNB suggests that estrogen receptors do not play a role in the development of the nucleus since flutamide is ineffective in blocking tritiated E uptake in rat uterine extract (DeBold et al., 1981; J. DeBold, personal communication). On the other hand, flutamide effectively blocks DHT uptake by receptors in the rat kidney and brain (DeBold et al., 1981). Hence the demasculinization caused by flutamide or its metabolites is probably due to interference with androgen but not estrogen action.

Inasmuch as prenatal FL has drastic consequences for the development of the SNB system, one may infer that those behaviors altered by FL treatment may involve this neuromuscular complex, and those behaviors unaffected by FL probably do not involve the SNB system. Therefore, traditionally defined mounting and intromission behaviors, which in the present study were unaffected by FL treatment, are probably not mediated by the SNB or its target muscles. In fact, all of the males receiving FL prenatally and oil postnatally displayed some mounting and intromission behavior, even though the majority of these males lacked the SNB target muscles. Since the SNB target muscles attach exclusively to the base of the penis, the SNB neurons undoubtedly control functions of the penis. However, such control need not be reflected in the gross postural movements associated with intromission in normal males and which, for convenience, are often equated with literal intromission of the penis. For example, the postural correlates of intromission are displayed by normal females given TP in adulthood (Ward, 1969), but these females do not possess a penis or the associated striated perineal muscles, and they are clearly not intromitting in a typically male fashion. Similarly, *tfm* male rats occasionally show intromission and even ejaculation-like behavior patterns (Olsen, 1979), even though they lack the SNB target muscles (Breedlove and Arnold, 1981). But if the BC/LA muscles are not essential for the display of the postural correlates of intromission, there is direct evidence of their importance to male fertility (Sachs, 1982) and penile reflexes (Hart and Melese-d'Hospital, 1981; Sachs, 1982). In other species, the BC muscle is active during literal erection and/or ejaculation (Kollberg et al., 1962; Hart, 1972; Beckett et al., 1975).

While the present results deny the importance of aromatized metabolites for the masculinization of the SNB system, the effectiveness of TP in masculinizing but the ineffectiveness of FL in demasculinizing mounting and intromission implies that aromatized androgens may be important for those functionally defined behaviors. It may be that FL has no effect on these behaviors because estrogen receptors within the CNS are not blocked by FL and are responsible for the masculinization of these behavioral correlates. On the other hand, it is still possible that androgen receptors contribute to the masculinization of these behaviors since Clemens et al. (1978) reported fewer mounts and intromissions by male rats given prenatal FL. The discrepancy between the results of Clemens et al. (1978) and the present study may be due to differences in the strains of rats used, dose of TP in adulthood, or age at which the males were castrated.

It appears that the sex differences in the SNB can be totally explained by androgen-induced changes during the perinatal period and that other, nonhormonally mediated mechanisms need not be involved. Such an idea was also suggested when *tfm* rats were found to have a feminine SNB system (Breedlove and Arnold, 1981). Since the only known defect in these animals is in their androgen receptors (Naess et al., 1976; Fox, 1975), it appeared that the only aspects of the genome affecting the SNB were those affecting hormonal action. Nonetheless, it is theoretically possible that there are other, nonhormonally related defects in the *tfm* genome which might be responsible for the feminine SNB. This possibility is made less likely by the present demonstration that hormonal manipulation by prenatal FL treatment combined with neonatal castration can completely demasculinize the SNB of rats with a normal male genotype.

The cross-fostering was intended to detect any postnatal demasculinization that might be caused by contamination of the milk supply of the dams treated with flutamide prior to delivery. We found no evidence of such contamination. Most measures, i.e., the number of SNB cells, SNB neuronal nuclear size, and number of LA fibers, were unaffected by cross-fostering. Unexpectedly, the two measures that were altered by cross-fostering actually resulted in a slight masculinization of PG-treated pups cross-fostered to a FL-treated dam. Specifically, SNB soma size is masculinized by cross-fostering regardless of the treatment of pups or dams. Perineal muscle complex weight was increased only in PG-treated pups cross-fostered to FL-treated dams. Therefore the relatively weak effects of cross-fostering are probably due to cross-fostering *per se*, independent of FL or PG treatment of the dam. Thus any flutamide leakage to a dam's milk supply is of little consequence to the masculinization of the SNB system. The fact that cross-fostering itself altered SNB soma size but not the number of SNB neurons implies that there are independent mechanisms for the masculinization of these two dimorphic aspects of the SNB (see Breedlove and Arnold, 1983).

The above results demonstrate that the SNB system is hormonally sensitive during both the prenatal and postnatal periods. Perinatal treatment of females with androgens also affirms this hormone sensitivity (Breedlove and Arnold, 1983). The mechanism whereby androgens masculinize the number of SNB neurons is presently unknown. However, the fact that postnatal manipulation can reduce the number of SNB cells in males may rule out the possibility that hormones alter proliferation of these neurons, since recent experiments in this laboratory have shown that male SNB cells are postmitotic by day 14 of gestation (Breedlove, et al., 1983). Similarly, the site at which androgen masculinizes the SNB may be the target muscles, which have androgen receptors in adulthood (Dube et al., 1976), or the SNB motoneurons themselves, which accumulate androgens (Breedlove and Arnold, 1980) and respond morphologically to TP in adulthood (Breedlove and Arnold, 1981).

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