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HORMONAL CONTROL OF A DEVELOPING NEUROMUSCULAR SYSTEM

II. Sensitive Periods for the Androgen-induced Masculinization of the Rat Spinal Nucleus of the Bulbocavernosus[1]

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Abstract

The spinal nucleus of the bulbocavernosus (SNB) and its target muscles are reduced or absent in normal female rats (Breedlove, S. M., and A. P. Arnold (1980) Science *210:* 564–566). We now report that prenatal treatment of females with testosterone propionate (TP) significantly increases the number of SNB neurons found in adulthood. Dihydrotesterone propionate (DHTP) treatment just after but not before birth also masculinizes the number of SNB neurons in females. SNB soma size is significantly masculinized, i.e., enlarged, by administration of androgen prenatally or as late as 7 to 11 days after birth, even though this late postnatal treatment has no effect on the number of SNB cells. Following TP treatment in adulthood, the androgenized females did not display the postural correlates of male copulatory behavior more often than did control females. From these results we infer the following. (*1*) Androgens act both before and after birth to influence the sexually dimorphic development of the SNB system. (*2*) There are different sensitive periods for the masculinization of SNB neuronal number and neuronal size, indicating that these two dimorphic characteristics of the SNB are masculinized by somewhat independent mechanisms. (*3*) TP and DHTP may act via separate mechanisms to alter the number of SNB neurons. (*4*) Aromatized metabolites of testosterone are not necessary for masculinization of the SNB system. (*5*) Virilization of the SNB system does not ensure the masculinization of the traditionally defined measures of male copulatory behavior in rodents.

Hormones are said to exert an organizational effect on the central nervous system (CNS) when a relatively brief exposure to the hormone during a critical period, usually around the time of birth, permanently alters the behavior or neuroendocrine function of the animal (Phoenix et al., 1959). There have been several recent descriptions of sexual dimorphisms in the CNS which indicate that perinatal hormones can organize the actual structure of the nervous system (Raisman and Field, 1973; Nottebohm and Arnold, 1976; Greenough et al., 1977; Gorski et al., 1978; Arnold and Saltiel, 1979; Jordan et al., 1982). These dimorphisms provide an opportunity to determine how hormones organize the CNS and how these alterations influence behavior (Jacobson and Gorski, 1981; Gurney, 1981). The exact nature of the organizational influences of hormones is still largely unknown (Arnold, 1980; A. P. Arnold and S. M. Breedlove, manuscript in preparation), but the temporal limits imposed by the critical period provide a salient clue to the mechanisms involved. Before one can identify the mechanism by which hormones alter CNS structure, one must determine when the hormone is effective.

We have previously described a sexually dimorphic motor nucleus in the rat lumbar spinal cord (Breedlove and Arnold, 1980). This spinal nucleus of the bulbocavernosus (SNB) is made up of motoneurons innervating the striated perineal muscles bulbocavernosus (BC) and levator ani (LA). The muscles are normally absent in adult female rats (Wainman and Shipounoff, 1941), unless they are given androgens shortly after birth (Cihak

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et al., 1970). The neurons of the SNB are about one-third as numerous and one-half as large in females as in adult males (Breedlove and Arnold, 1980, 1981). Adult hormone manipulation does not alter the number of SNB neurons, but the size of these neurons is sensitive to adult androgen exposure (Breedlove and Arnold, 1981). This latter effect of hormone administration in adulthood can be viewed as an activational hormonal influence. Thus there are at least two independent measures of the masculinity of the SNB: neuronal number, which seems to be influenced only by organizational androgen action, and neuronal size, which is subject to both organizational and activational androgen effects. Previous reports documented the hormone accumulation (Breedlove and Arnold, 1980) and morphological response of SNB neurons to androgen (Breedlove and Arnold, 1981). The present experiment delineates the sensitive periods for androgenic organization of both the number and the size of SNB neurons and the number of target muscle fibers. We find that the critical period for the organizational masculinization of SNB cell size extends beyond the critical period for masculinization of SNB number. Furthermore, the target muscles of the SNB can be masculinized independently of the SNB motoneurons. Finally, the perinatal masculinization of the SNB system does not ensure increased masculine sexual behavior in response to androgen in adulthood.

Materials and Methods

After female rats displayed at least two regular 4-day estrous cycles, they were left in a group cage with stud males on the day of proestrus. If sperm was seen in the vaginal smear the following day, this day was designated day 1 of gestation. All dams delivered their litters on day 23 of gestation, which was considered postnatal day 1. From day 1 until day 10 of gestation, pregnant dams were gently handled each day. Rat pups were treated with hormone during one of four developmental periods. Prenatal hormone was delivered by subcutaneous injections of the pregnant dam with 2 mg of testosterone propionate (TP) or dihydrotestosterone propionate (DHTP) daily from days 11 to 16 of gestation (early prenatal), or from days 17 to 22 of gestation (late prenatal). Postnatally, 1 mg of TP or DHTP was injected subcutaneously into the pups on three alternate days: either days 1, 3, and 5 (early postnatal), or days 7, 9, and 11 (late postnatal). When pups were injected, the needle puncture was sealed with flexible collodion. On the day of birth all treated female pups were ovariectomized and marked individually by toe clipping while anesthetized by cooling. There were three control groups of each sex. Control male and female rats were either (*a*) gonadectomized at birth and oil treated, (*b*) sham operated and oil treated, or (*c*) not disturbed with neonatal treatment. With the exception of the perinatally undisturbed control groups, all pups were injected at each of the developmental periods, either with hormone or oil, in order to equalize as much as possible the stress of perinatal manipulations. Pups exposed to late prenatal TP, for example, received oil injections during the other periods.

On the day of birth the ano-genital distance (AGD) for each pup was measured. While the AGD at birth of hormonally treated females was sufficiently masculinized to make sex determination of the pups difficult by this method, the appearance of the gonads at birth was not altered by any prenatal treatment. Therefore, except for those control animals that were undisturbed perinatally, the sex of animals was determined by inspection of the gonads during the castration or sham surgery on the day of birth. Prenatally androgenized females were cross-fostered to dams which had received only oil injections. Each dam was left with eight pups. Rats that were sham operated at birth were gonadectomized on day 40, while those rats gonadectomized at birth were sham operated on day 40.

All pups were weaned on day 21 of life and housed in same-treatment groups of two to four. 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. This adult androgen treatment provided an equivalent "activational" background from which organizational effects of perinatal hormones could be detected. Androgens cause an activational enlargement of SNB somas in adult rats (Breedlove and Arnold, 1981). The masculine copulatory behavior of all animals was tested beginning 22 days after androgen treatment commenced. Testing procedures are described in the previous report (Breedlove and Arnold, 1983).

Animals were sacrificed with sodium pentobarbital and were perfused intracardially with saline followed by buffered 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 (IC) and the base of the penis to which these muscles attach, was also removed and postfixed. Spinal cords were frozen and sectioned 50 μm thick, and alternate sections were mounted and stained with thionin. The number and size of SNB neurons were estimated as described previously (Breedlove and Arnold, 1981, 1983). 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 LA was taken to provide an estimate of the number of muscle fibers as described in Breedlove and Arnold (1983).

Statistical analyses consisted of repeated t tests comparing androgenized females to the neonatally gonadectomized, oil-treated female controls. Because there were six experimental groups to evaluate, a p of 0.01 rather than 0.05 was selected to reject the null hypothesis. Because androgens were predicted to masculinize the SNB and its target muscles, a one-tailed p value was used. Of the available animals, eight from each treatment group were randomly chosen for analysis, except for the gonadectomized, oil-treated female control group, which had an N of 7. These neonatally gonadectomized, oil-treated females served as the control group for all comparisons involving androgenized females. The nonparametric Mann-Whitney U test, with a p value adjusted as above, was used for comparing latency measures.

Results

Fetus and pup survival. The perinatal treatments significantly hindered the birth and subsequent survival of

rats. Androgen treatment of dams during the early prenatal period prevented delivery, with the exception of only one dam receiving early prenatal DHTP. This dam delivered only four female pups which were insufficient to form an experimental group. Therefore, there are no experimental groups of females receiving early prenatal androgen. Prenatal treatment with TP during the last week of pregnancy resulted in only 63% of the dams delivering live young compared to 96% of oil-injected control dams. Of dams injected with DHTP during the late prenatal period, 67% gave birth. Of pups born alive, 8 of 25 of those exposed to late prenatal TP did not survive until sacrifice at days 101 to 103. Of the live-born pups given DHTP late prenatally, 11 of 22 died before sacrifice. Some of these postnatal deaths may not be due to the androgen treatment, since 7 of 18 gonadectomized female controls died and 2 of 10 sham-operated female controls died before sacrifice at days 101 to 103. Thus the perinatal manipulations were undoubtedly stressful, independent of the androgen treatment *per se*. Therefore the neonatally gonadectomized, oil-treated females are the proper control group for comparisons of the androgenized females which were also gonadectomized at birth.

Number of SNB neurons. Only two perinatal hormone treatments significantly masculinized, i.e., increased, the number of SNB neurons seen in adulthood: prenatal TP and early postnatal DHTP ($p < 0.01$, one tailed, compared to the neonatally gonadectomized, oil-treated female controls; Figs. 1 and 2). Early postnatal TP increased the number of SNB cells, though not significantly. Prenatal DHTP actually resulted in fewer, although not significantly fewer, SNB cells than in neonatally gonadectomized female controls. There were no significant differences in the number of SNB cells among female control groups. In this study, day 1 castration of males did not significantly reduce the adult number of

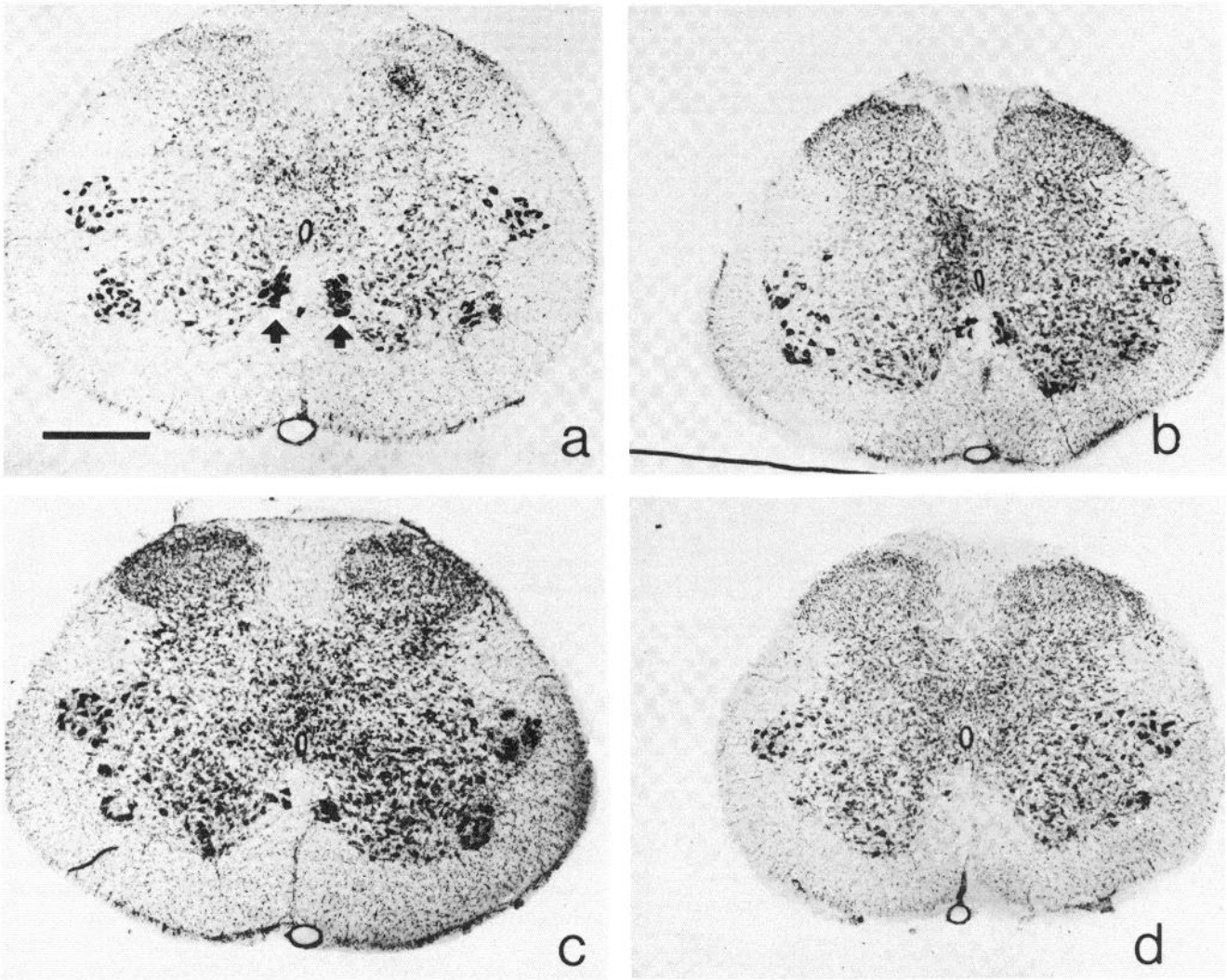

Figure 1. Photomicrographs of the fifth lumbar segment of the adult rat spinal cord in the region of the spinal nucleus of the bulbocavernosus (SNB). *a*, The *arrows* point to the densely staining neurons of the SNB in a control male (sham operated at birth). *b*, Prenatal treatment of females with testosterone propionate results in more and larger SNB cells in adulthood. These females, unlike normal female rats, have the masculine striated muscles innervated by the SNB of males. *c*, Females given dihydrotesterosterone propionate just after birth also have more and larger SNB cells, as well as their target muscles in adulthood. *Scale bar*, 500 μm. *d*, Such cells are smaller and more scarce in oil-treated female controls ovariectomized at birth.

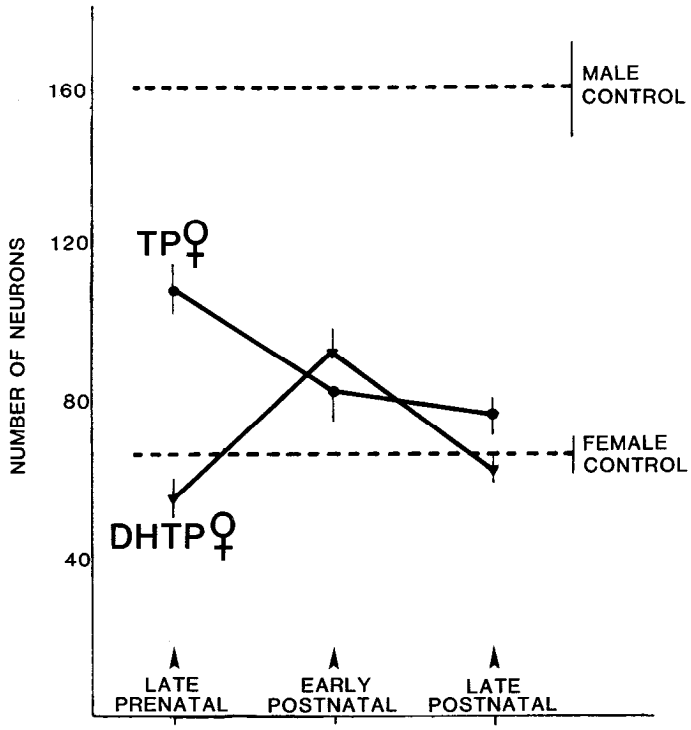

Figure 2. Perinatal androgen administration significantly masculinizes the adult number of neurons in the spinal nucleus of the bulbocavernosus (SNB). DHTP administered during the early postnatal period masculinized the number of SNB cells. TP given prenatally also increased the adult number of SNB neurons. The graph represents the mean ± standard errors of the means, using $N = 8$ animals per experimental group.

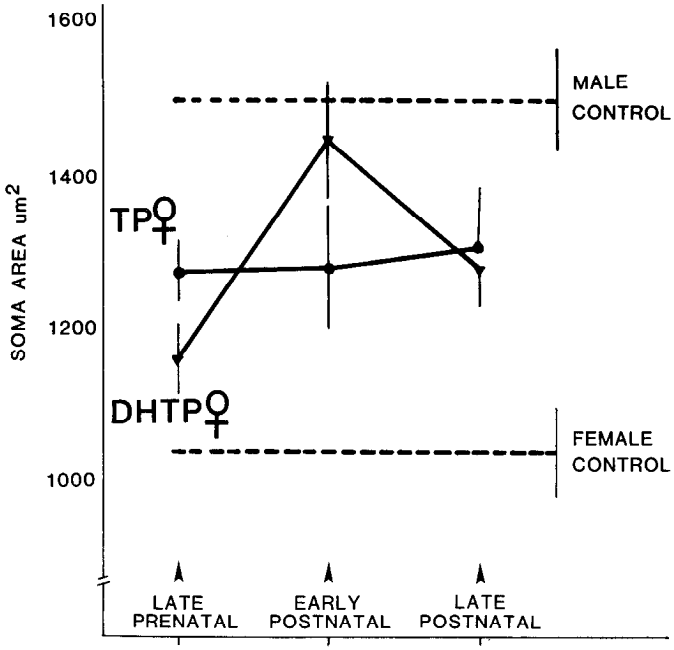

Figure 3. Perinatal androgens significantly masculinize the size of SNB neuronal somas found in adulthood. Only prenatal DHTP failed to significantly increase SNB cross-sectional area in adulthood. This effect of androgens is apparently organizational, since all of the animals represented above were gonadectomized either on the day of birth or, in the case of the male controls, in adulthood, then treated for 33 days with TP.

SNB neurons relative to neonatally sham-operated males, but both of these groups had significantly fewer SNB cells than did the perinatally undisturbed males.

Cross-sectional area of SNB neurons. All perinatal androgen treatments except late prenatal DHTP resulted in significantly larger somas (Fig. 3) and nuclei (Table I) of SNB cells. There were no effects of gonadectomy on SNB cell size in either the male or the female control groups.

Perineal muscle weights. The adult female rat does not normally have the muscles BC, LA, and IC, nor did any of the control females in this study. Females receiving perinatal androgen, however, had all three of these muscles, except for five of the eight females in the late postnatal DHTP group. These five late postnatal DHTP females without the muscles did not have fewer or smaller SNB cells than the three late postnatal DHTP females with the muscles. The weights of the perineal muscle complex are displayed in Table II. Among the control males, the sham-operated and undisturbed males had equally heavy muscles, but neonatally gonadectomized males had significantly lighter muscles (Table II, legend).

Number of levator ani muscle fibers. Once again, excepting the females treated with DHTP in the late postnatal period (see above), androgenized females had significant numbers of LA muscle fibers (Fig. 4), and none of the control females had any. There were no significant differences in the number of LA fibers in any of the male control groups. This is in contrast to the significant decrease in muscle complex weight in the neonatally castrated males, as described above.

TABLE I

The cross-sectional area, in square micrometers, of the nuclei of cells in the SNB

Values shown are the mean ± SEM size of SNB neuronal nuclei from females exposed to either TP or DHTP during one of three perinatal periods. $N = 8$ animals per group. The size of nuclei in neonatally gonadectomized, oil-treated control females was 161.9 ± 9.5 ($N = 7$) μm^2. The SNB nuclei of neonatally sham-operated female controls were 157.1 ± 10.6 μm^2, while those of neonatally undisturbed females were 185.1 ± 8.0 μm^2. Neonatally gonadectomized males had 245.4 ± 9.1, neonatally sham-operated males had 246.2 ± 10.0, and neonatally undisturbed males had 244.6 ± 6.4 μm^2 nuclei.

	Late Prenatal	Early Postnatal	Late Postnatal
TP	209.1 ± 9.3	208.8 ± 6.6	218.0 ± 13.4
DHTP	196.0 ± 12.2	210.3 ± 13.4	205.4 ± 12.4

Masculine copulatory behavior. All of the females displayed mounting behavior, and most of them displayed intromission at least once. However, there were no significant differences between any perinatally androgenized female groups and the oil-treated, neonatally ovariectomized control females for mounting or intromission behavior (Table III). None of the females displayed any ejaculatory behavior. Apparently, the perinatal manipulations, independent of the hormone treatment, masculinized the behavior of females somewhat, since the perinatally undisturbed female controls waited significantly longer to mount (mean, 265.1 sec), compared

TABLE II

The weight, in grams, of the perineal muscle complex taken from perinatally androgenized female rats

All of these females had all three striated perineal muscles, with the exception of five of the eight late postnatal DHTP females. $N = 8$ animals in each group. None of the control females had any of the three muscles comprising the complex. Of the three late postnatal DHTP females that had the muscles, the mean perineal weight was 0.54 ± 0.06 gm ($N = 3$). Male control weights were: perinatally undisturbed males, 2.15 ± 0.05 gm ($N = 8$); neonatally castrated, oil-treated males, 1.29 ± 0.15 gm ($N = 8$); and neonatally sham-operated, oil-treated males, 2.19 ± 0.06 gm ($N = 8$).

	Late Prenatal	Early Postnatal	Late Postnatal
TP	0.77 ± 0.08	0.79 ± 0.05	0.77 ± 0.08
DHTP	0.38 ± 0.04	0.66 ± 0.02	0.20 ± 0.10

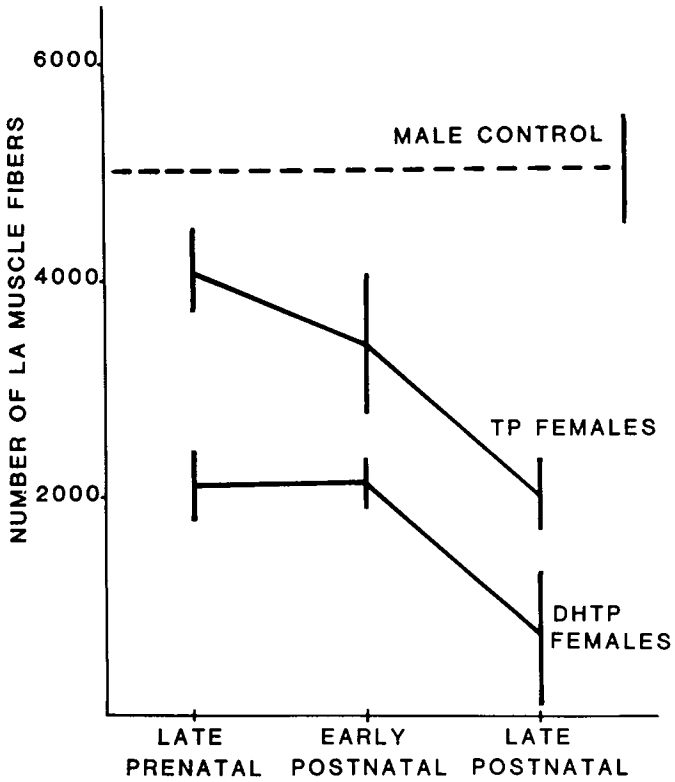

Figure 4. Perinatal androgen treatment significantly masculinizes the adult number of muscle fibers in the levator ani (LA) muscle in female rats. Adult female rats normally have no LA fibers. Thus, of the treatment groups (*base* of figure), only late postnatal DHTP treatment failed to significantly increase the number of LA fibers in adulthood. The means shown (*left*) are based on $N = 8$ animals per group, but only 3 of the late postnatal DHTP females possessed LA muscles.

to either neonatally ovariectomized (mean, 101.2 sec) or sham-operated (mean 155.8 sec) female controls ($p < 0.01$, Mann-Whitney U test). Among the male control groups, the neonatally sham-operated and the perinatally undisturbed males displayed equivalent numbers and latencies for mounting and intromission (Table III, legend). The only significant difference between these control males was in the mean number of ejaculations ($p < 0.01$, t test; means given below). The neonatally castrated males, on the other hand, displayed significantly fewer intromissions, more mounts without intromission, and therefore a lower intromission efficiency than either male

TABLE III

Mean number of behaviorally defined intromissions per 20-min test session in perinatally androgenized female rats

N = 8 animals per group. Values for control females were: perinatally undisturbed, 0.8 ± 0.27; neonatally sham-operated, 2.4 ± 0.74; neonatally ovariectomized, 1.5 ± 0.60. Values for control males were: perinatally undisturbed, 18.7 ± 0.77; neonatally sham-operated, 20.0 ± 1.79; neonatally castrated, 2.9 ± 1.75.

	Later Prenatal	Early Postnatal	Late Postnatal
TP	1.6 ± 0.73	5.0 ± 1.58	2.9 ± 1.35
DHTP	2.1 ± 0.61	1.9 ± 0.36	1.1 ± 0.34

control group. Only one of the eight neonatally castrated males ever ejaculated, while all of the other males displayed at least two and usually more than four ejaculations during total testing (mean ± SEM: neonatally castrated males, 0.19 ± 0.19; neonatally sham-operated males, 1.7 ± 0.11, vs. perinatally undisturbed males, 1.1 ± 0.15 ejaculations per session). For all measures of copulatory behavior, the neonatally castrated males were statistically indistinguishable from neonatally ovariectomized female controls.

Ano-genital distance (AGD) at birth. The AGD of pups at birth confirmed the effect of prenatal androgen. Female pups exposed to prenatal oil had a mean AGD of 2.5 ± 0.05 (SEM) mm $N = 45$), while oil-treated males had a mean AGD of 4.6 ± 0.12 mm ($N = 48$). The perinatally androgenized females had a mean AGD intermediate to these: prenatal TP females had a mean AGD of 3.8 ± 0.07 mm ($N = 17$) and prenatal DHTP females had a mean AGD of 3.2 ± 0.08 mm ($N = 21$).

Discussion

There are several dimensions of the SNB system which are sexually dimorphic: the size of SNB neurons, the number of SNB neurons, and the number of target muscle fibers. Both prenatal and postnatal androgen treatment successfully masculinized each of these aspects of the SNB system. Androgens were administered during several developmental periods in order to delineate the critical period for masculinization of the SNB system. In fact, two critical periods were revealed. The finding that late postnatal treatment with either androgen significantly masculinized the soma size of SNB neurons without altering their number indicates that these two characteristics are masculinized during different, albeit overlapping, critical periods. The critical period for the masculinization of SNB neuronal number begins prenatally and seems to end by about postnatal day 5. The end of the critical period for the masculinization of SNB neuronal size was not defined by the present study, but extends beyond postnatal day 5. Yet there must be an end to the critical period for SNB neuronal size, since a 32-day regimen of high TP doses just preceding sacrifice of these females could not erase the SNB size differences between perinatally androgenized females and controls. Thus the mechanisms responsible for organizational masculinization of the number and size of SNB neurons are somewhat independent.

There was a discrepancy between the effects of TP and DHTP on the number of SNB cells. TP most effectively increased the number of SNB neurons when deliv-

ered during the week before birth, while DHTP was ineffective during this period. Perhaps the simplest explanation of this disparity is that DHTP reached the crucial site or sites less efficiently than TP. The increased number of SNB neurons resulting from postnatal DHTP indicates that such an explanation could apply to the fetus but not the newborn. Thus, there may be inefficient transport of DHTP to the fetus, or rapid conversion of DHTP injected into the dam to another, non-masculinizing metabolite. However, at birth, the AGD of prenatal DHTP females was significantly greater than that of control females, indicating that DHTP was indeed reaching the fetuses. Similarly, the females treated prenatally with DHTP had the perineal muscle targets of the SNB, which were as masculinized as those of females treated with DHTP in the early postnatal period (Fig. 3), indicating that the SNB target muscles responded to DHTP or its metabolites prenatally. This discrepancy in the maximally effective period of the two androgens, along with the fact that DHTP, but not TP, masculinizes the neurons and targets differentially, implies that the two androgens can affect the SNB system via different mechanisms. These mechanisms might involve different active metabolites, receptors, or sites of action of the two androgens. Since TP and DHTP differentially hasten reinnervation of muscle by axotomized hypoglossal motoneurons (Yu and Srinivasan, 1981), it is possible that the two androgens differentially enhance SNB axonal outgrowth during development.

The late prenatal DHTP treatment masculinized the SNB target muscles but not the SNB motoneurons themselves. This independence of effect on neuron and target may be important to understanding the way each is masculinized. Apparently, masculinization of the muscles does not require masculinization of the SNB. There are at least four possible explanations of how late prenatal DHTP treatment could result in more SNB target muscle but no more SNB motoneurons. Either (*a*) there are uninnervated muscle fiber; (*b*) the extra muscle fibers are innervated by cells which, unlike all other known CNS motoneurons, are small and lightly Nissl-staining; (*c*) the number of muscle fibers innervated per motoneurons is increased; or (*d*) there are additional motoneurons which are not recognized because they are outside their normal anatomical locus in the SNB. The first two of these possibilities seem unlikely since preliminary HRP experiments in similarly androgenized females indicate that the resulting BC/LA muscles are indeed innervated by spinal cells which meet the classic morphological requirements for motoneurons, i.e., large, multipolar, densely Nissl-staining somas. Thus the other two explanations are more plausible. Androgens might alter the ratio of motoneuron to muscle fibers by altering the transfer of trophic factor(s), such that each motoneuron maintains (or is maintained by) more muscle fibers. Androgens might affect which motoneurons innervate these particular muscles by altering chemical recognition between the two, by altering environmental cues that guide axons to the proper muscle, or by augmenting the outgrowth of particular motoneurons (see Yu and Srinivasan, 1981), and thereby grant them a pioneer's advantage for innervation.

How do perinatal androgens result in more adult SNB neurons? There are three possible methods which are not mutually exclusive. (*1*) Androgens might increase the mitotic activity of SNB neuroblast precursors, resulting in more SNB neurons in males. (*2*) Androgens might inhibit the naturally occurring motoneuronal death resulting in more surviving SNB neurons in males. (*3*) Androgens might alter the "specification" of developing cells so that they develop into SNB neurons in males rather than some other type of CNS cell in females. The first of these possibilities now seems unlikely since SNB cells are postmitotic by day 14 of gestation (Breedlove et al., 1983), yet a week later, postnatal DHTP treatment of females results in more SNB neurons, and neonatal castration of males can result in fewer SNB cells (Breedlove and Arnold, 1983). At present it seems most likely that androgens inhibit motoneuronal death around the time of birth of the rat. The question of whether androgens masculinize spinal and perineal morphology by acting primarily on the motoneurons or their muscle targets remains open. The report of Cihak et al. (1970) that the LA is present at birth in female rats, but involutes in the first few weeks of life, occasionally after developing cross-striations, implies that at least some of these degenerating muscles are innervated. Thus perinatal androgen may preserve the muscles by altering the activity or trophic factors from either the neurons or the muscles.

It is interesting that in the present study, females can be significantly masculinized by postnatal exposure to androgen, but males are not quite significantly demasculinized by neonatal castration. This may be either because the castration of males on day 1 took place too late in this study to prevent postnatal androgenization, or because there is an interaction between pre- and postnatal androgen exposure. More specifically, the presence of androgen during either the prenatal or postnatal period may significantly compensate for a lack of androgens during the other period. Such a compensatory mechanism is supported by another study (Breedlove and Arnold, 1983) in which prenatal delivery of the antiandrogen flutamide (FL), in conjunction with day 1 castration, causes a complete demasculinization of the SNB system. However, if either the prenatal androgen blockade with FL is omitted or the postnatal androgen deprivation is circumvented by TP therapy, the SNB system is very significantly masculinized. This compensation hypothesis implies that the SNB system is very sensitive to initial androgen exposure, no matter whether this exposure is prenatal or postnatal, but becomes less sensitive thereafter. Such an effect might be due to a decreased hormone sensitivity. Alternatively, it may be due to a "ceiling effect," in which the initial androgen exposure masculinizes the system nearly completely, allowing little room for further masculinization by subsequent androgen.

The two muscles innervated by the rat SNB, the BC and LA, are both homologous to the muscle called bulbocavernosus in other mammals (Hayes, 1965). Therefore, the rat SNB is functionally homologous to Onuf's nucleus in other mammals, since Onuf's nucleus innervates the BC in cats (Yamamoto et al., 1978) and humans (Onufrowicz, 1899). Schroder (1981) makes the case that

the rat SNB (which he labels the dorsal medial nucleus) is also anatomically homologous to Onuf's nucleus. If the rat SNB and human Onuf's nucleus are indeed homologous, then one would expect to find more and larger neurons in the male human Onuf's nucleus than in the female. However, there are no known perineal muscles which are present in only one sex in humans. Thus a sexual dimorphism in human Onuf's nucleus might be less pronounced than that of the rat SNB.

Onuf's nucleus was recently reported to be spared in human patients suffering amyotrophic lateral sclerosis (ALS; Mannen et al., 1977), a disease that is characterized by massive motoneuronal death. Interestingly, Weiner (1980) recently proposed that motoneurons that heavily accumulate androgens are more susceptible to ALS. Based on this reasoning, Weiner (1980) predicted that the rat homologue to Onuf's nucleus, which includes the SNB (see Schroder, 1981), would not accumulate androgens heavily. In fact, virtually all SNB neurons accumulate androgens and do so more heavily than other motoneurons in the same spinal segments (Breedlove and Arnold, 1980).

Androgenized females seemed to have either the three striated perineal muscles or none. When the muscles were present, they were large enough and their anatomical configuration was masculine enough to make them unmistakable. Thus there may be a "quantal" nature of perineal muscle development in rats, such that a critical number of fibers or some other factor must be present for the muscle to persist. The fewest number of LA fibers seen in the present study was 710 fibers in a late postnatal DHTP female. It may be that about 700 LA fibers constitute a quorum for muscle development. Another indication that the presence of the LA is a binary event is the observation of occasional androgenized females which had the LA on one side only. This remarkable condition was seen in two females, one with only a right LA and one with only a left LA. Both of these females had received early postnatal DHTP. Unfortunately, the spinal cords were sectioned before the condition of the LA was discovered and no provisions were made to distinguish the left and right halves of the spinal cord. The number of muscle fibers in these unilateral LA muscles was 1583 and 1120. Among their treatment group, these animals had the fewest estimated unilateral LA muscle fibers.

Because DHTP does not convert readily to testosterone (T) or estradiol (E), the effectiveness of postnatal DHTP on the spinal cord implies that aromatization of T to E is not necessary for masculinization of the SNB. This finding contrasts with previous evidence that the conversion of T to E is required for the masculinization of the brain, as detected by the pattern of gonadotropin release (Gorski and Wagner, 1965), lordosis (Christensen and Gorski, 1978), masculine copulatory behavior (Sodersten and Hansen, 1978), or morphological measures (Jacobson, 1980). The SNB seems to be an exception to the rule that aromatized metabolites of T masculinize the CNS. Furthermore, the SNB undoubtedly receives afferents from other parts of the nervous system, and the virilization of the SNB by non-aromatized steroids could be expected to alter the development of these regions, at least indirectly. If this "domino effect" takes place, then DHTP could mediate the sexual differentiation of other CNS sites, providing additional exceptions to the aromatization rule.

The failure of perinatal androgen exposure to masculinize adult copulatory behavior is not unprecedented. While there are several reports of early TP treatment of females augmenting such behaviors in adulthood (Harris and Levine, 1965; Gerall and Ward, 1966), this organizational masculinization is not always seen for mounting (Whalen and Edwards, 1967; Hendricks, 1969) or intromission (Whalen et al., 1969; Huffman and Hendricks, 1981). None of these authors reported ejaculatory behavior in their androgenized female rats. These inconsistent results contrast with the drastic demasculinization of males castrated on the day of birth in this and other studies (Beach and Holtz, 1946; Grady et al., 1965). Ward (1969) suggested that both prenatal and postnatal androgenic stimulation is necessary to masculinize female rats. Ward (1969) found that 80% of females exposed to androgen both before and after birth displayed ejaculation at least once during rather extensive testing, but only 20% of the exclusively postnatally androgenized and none of the prenatally androgenized females ever ejaculated during 15 weekly tests. Parenthetically, Ward's androgenized females, like those of the present study, displayed no more mounting or intromission than did control females. More exhaustive tests in the present study might have revealed the ejaculatory potential of some of the androgenized females, and thus it is still possible that the SNB system is involved in ejaculation. This possibility is partially supported by the fact that prenatally administered anti-androgen inhibits the development of both the SNB system and ejaculation in male rats (Breedlove and Arnold, 1983). On the other hand, the fact that normal females, without SNB target muscles, mount and intromit as much as females with a considerably well developed SNB system implies that the SNB and its target muscles do not play a role in these behaviors. Similarly, male testicular feminized (*tfm*) rats without SNB target muscles (Breedlove and Arnold, 1981) sometimes show intromission and ejaculatory behavior patterns (Olsen, 1979).

It should be pointed out, however, that the present and previous studies utilize a rather restrictive definition of male copulatory behavior. Mounting is unmistakable but is not very sexually dimorphic (see Beach, 1971). Intromission, in contrast, is typically defined in terms of the postural correlates of intromission. Specifically, intromission is defined as the rather distinctive behavior a normal male displays during intromission; that is, a thrusting mount ending with a backward springing dismount. Because these behavioral concomitants of intromission are displayed by animals who have no penis (i.e., normal females treated with TP in adulthood), they are certainly not equivalent to literal intromission of the penis, and they are probably not directly relevant to the activity of the perineum. Thus these postural measures exclude a great deal of masculine copulatory activity which might be mediated by the SNB or its muscles. In confirmation of this suggestion, other researchers have recently reported that lesions of striated perineal muscles

in rats decrease penile reflexes (Hart and Melese-d'Hospital, 1981) and fertility, without altering the postural concomitants of male behavior (Sachs, 1982). Studies in other species find that the BC muscle is active during literal erection and/or ejaculation (Kollberg et al., 1962; Hart, 1972; Beckett et al., 1975; Purohit and Beckett, 1976). Thus there is considerable evidence that the muscles controlled by the SNB are important to rat male sexual behavior but are probably not mediating mounting or posturally defined intromission. The fact that *tfm* males without these target muscles show postural correlates of mounting, intromission, and ejaculation (Olsen, 1979) indicates that the muscles are not crucial for such postures.

The significant mortality caused by perinatal administration of hormone suggests that these doses had a toxic effect on the rats. However, there was also significant mortality among the female controls that received no hormone, suggesting that nonendocrine factors might account for some deaths. Nevertheless, a failure of an androgen treatment to masculinize either CNS morphology or behavior could be due to a general debilitating effect. If, on the other hand, a hormone treatment masculinizes the SNB despite putative toxic side effects, one can be reasonably certain that these effects are due to specific hormone action. It would not seem reasonable that a general toxic effect could *increase* the number of surviving SNB neurons or LA muscle fibers.

Treatment of female pups with TP just after birth increased the number of SNB cells, but not significantly. In a previous study, a single injection of TP did significantly masculinize the number of SNB neurons (Breedlove et al., 1982). The disparate results in the two studies can probably be understood by comparing the control groups in each case. In the previous study, female controls were subjected to a single oil injection on day 2 of life, while in the present study, control females came from mothers that had received repeated subcutaneous injections of oil, and the pups themselves received six injections postnatally. Furthermore, the control (and experimental) females in the present study were ovariectomized and some of their toes were amputated on the day of birth. These obviously stressful experiences might trigger release of adrenal hormones, which may have a masculinizing effect. Indeed, the control females from the present study did have more SNB neurons (66.6 ± 4.5, $N = 7$) than the control females from the previous study (44.2 ± 1.7, $N = 8$). Therefore, the lack of a statistically significant masculinizing effect on the number of SNB neurons with postnatal TP in the present study is probably due to a high base line for controls.

In conclusion, the sexually dimorphic SNB system is masculinized by androgens during both the prenatal and immediate postnatal periods. Non-aromatizable androgens are capable of inducing each of the masculinizing effects. There are many dissociations in the hormonally mediated masculinzation of the SNB system. The number and size of SNB neurons are affected independently. The number of SNB cells and the number of their target muscle fibers are independent. There may be two mechanisms determining the number of SNB neurons, since TP and DHTP seem to have their maximum effects at different developmental periods. These dissociations may prove useful in discerning the developmental mechanisms mediating the masculinization of the various components of the SNB system.

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