on, and have larger areas of rough endoplasmic reticulum, when compared to larger homologous cells from related species (*19*). Basal metabolic rate is associated with differences in relative brain size and has been suggested as a controlling factor in brain evolution (*3, 4*), although no functional or genetic cause has been proposed (*4*).
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25. Body weight of the mice was recorded at ten weekly intervals between day 14 and day 70. In rats, body weight was measured at intervals between day 14 and day 189. Brain weight was estimated as cranial volume of rats at 189 days and mice at either 38 or 70 days. After logarithmic transformation of all data and correction for sex differences, weight gain prior to 14 days was estimated as 14-day weight. Gain during subsequent intervals was computed as the difference between the logarithms of weights at the beginnings and ends of the intervals. The cross-fostering design allowed estimation of genetic, maternal, and residual environmental portions of variance and covariance (*11*).
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Androgens Prevent Normally Occurring Cell Death in a Sexually Dimorphic Spinal Nucleus

Abstract. *The spinal nucleus of the bulbocavernosus (SNB) contains many more motoneurons in adult male rats than in females. Androgens establish this sex difference during a critical perinatal period, which coincides with normally occurring cell death in the SNB region. Sex differences in SNB motoneuron number arise primarily because motoneuron loss is greater in females than in males during the early postnatal period. Perinatal androgen treatment in females attenuates cell death in the SNB region, reducing motoneuron loss to levels typical of males. The results suggest that steroid hormones determine sex differences in neuron number by regulating normally occurring cell death and that the timing of this cell death may therefore define critical periods for steroid effects on neuron number.*

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Sex differences in the adult vertebrate nervous system can include dramatic dimorphisms in the number and morphology of neurons devoted to various sexually dimorphic behaviors. Gonadal steroids produce many of these sex differences by acting during early "critical" periods to determine the number and size of neurons, as well as their neuritic arborization and biochemical characteristics (*1*). It is generally thought that steroids influence sexual differentiation by acting on developmental events that coincide with these critical periods. Little is known, however, about which developmental events are susceptible to steroid modulation. In this report, we describe the development and androgenic regulation of sex differences in motoneuron number within a rat spinal nucleus. We have found that the critical period during which androgens influence motoneuron number within this nucleus coincides with the period of normally occurring cell death. Moreover, androgens attenuate this cell death, permanently increasing the number of motoneurons retained to adulthood.

The spinal nucleus of the bulbocavernosus (SNB) is a discrete group of motoneurons located in the lumbar spinal cord. Adult male rats have approximately 200 SNB motoneurons innervating two penile muscles—the bulbocavernosus (BC) and levator ani (LA) (*2*)—as well as the anal sphincter (*3*). The LA-BC complex controls penile reflexes important in copulatory behavior (*4*). Adult females have only about 60 SNB motoneurons, and although the LA-BC complex is present in females during early development, these muscles atrophy during the first few weeks of postnatal life (*2, 5*). SNB motoneurons and their target muscles accumulate androgens in adult rats, and the masculinization of this neuromuscular system depends on the presence of androgens around the time of birth (*2, 6*). Males deprived of androgens perinatally have a female number of motoneurons as adults and lack the LA-BC complex. Conversely, females given androgens during an early critical period have an increased number of SNB motoneurons and retain the LA-BC complex to adulthood (*7*).

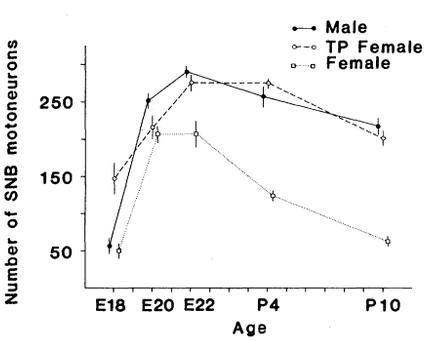
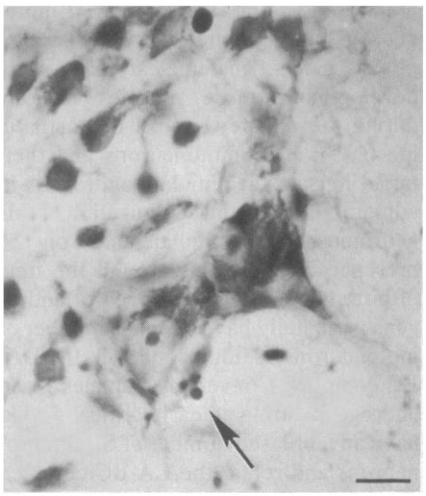


Fig. 1. Counts of SNB motoneurons from E18 through P10 for females (n = 24), TP females (n = 27), and males (n = 23). Points represent means ± standard errors; n = 3 to 7 per data point.

There are several mechanisms by which androgens could regulate SNB motoneuron number. One is that androgens might enhance the proliferation of SNB motoneurons. This possibility is unlikely, since virtually all SNB motoneurons undergo their last mitotic division on day 12 of gestation, before androgens are secreted by the testes and several days before sex differences in circulating androgens are apparent (*8, 9*). Moreover, if females are treated with androgens during the early postnatal period, their masculinized SNB also consists of motoneurons generated on embryonic (E) day 12, well before androgen treatment was begun (*10*). A second possibility is that androgens regulate SNB motoneuron number by influencing cellular migration or differentiation. In this case, androgens could either promote the differentiation of SNB motoneurons or their migration into the SNB region, or prevent their redifferentiation or migration out of the SNB. A final possibility is that androgens enhance the survival of SNB motoneurons during the period of normally occurring motoneuron death. We have focused on the latter two hypotheses in examining SNB ontogeny in males, females, and androgen-treated females during the critical period of androgenic action.

From E16 through E22 (*11*), pregnant Sprague-Dawley rats (Simonsen) either received subcutaneous injections of testosterone propionate (TP) (2 mg/day) dissolved in sesame oil or were left untreated. Pups born to TP-treated dams were cross-fostered to other lactating females and injected subcutaneously with 1 mg of TP on postnatal (P) day 1, P3, and P5. Male, female, and androgen-treated female (TP female) pups were killed with an overdose of pentobarbital sodium and perfused with saline formaldehyde on E18, E20, E22, P4, or P10. Lumbar spinal cords were fixed in Bouin's solu-



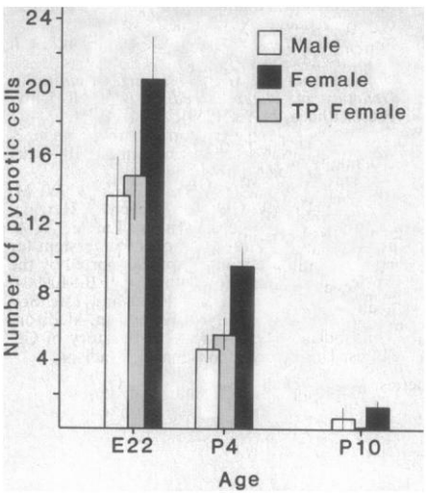


Fig. 2 (left). Photomicrograph of the SNB at E22 showing several normal motoneurons. Arrow, degenerating cell profile. Calibration bar, 10 μm. Fig. 3 (right). Degenerating cells at E22, P4, and P10 for females (n = 15), TP females (n = 16), and males (n = 15). Bars represent means ± standard errors; n = 3 to 7 per data point. Over all ages, females had significantly more degenerating cells in the SNB than either TP females or males.

tion, embedded in paraffin, serially sectioned (10 μm), and stained with cresyl echt violet. All SNB motoneurons with distinct nucleoli were counted, and these counts were corrected for split nucleoli (*12*). SNB motoneurons were easily recognized at all ages by their characteristic location within the cord and their large, densely staining, multipolar cell bodies (*2*).

Counts of SNB motoneurons during the perinatal period revealed two distinct phases in SNB development (Fig. 1). The first phase occurred between E18 and E22 and was characterized by a rapid increase in motoneuron number. At E18, all groups had relatively few motoneurons within the SNB region. No sex difference in SNB motoneuron number was apparent at E18, but TP females had significantly more motoneurons in the SNB than either normal males or females [F's(1, 59) > 19.00, P < 0.01] (*13*). Motoneuron number increased dramatically in all three groups between E18 and E22. This increase was slightly greater in males and TP females than in normal females, resulting in small, but significant, group differences in motoneuron number as early as E20 [F(1, 59) = 7.33, P < 0.01].

The second phase of SNB development occurred during the early postnatal period and was characterized by a significant loss of SNB motoneurons in all three groups [F's(1, 59) > 11.00, P < 0.01]. This loss was much greater in females than in males and could be attenuated in females by perinatal androgen treatment. Between E22 and P10, females lost nearly 70 percent of their SNB motoneurons. During this same period, males and TP females lost approximately 25 percent of their SNB motoneurons—considerably less than normal females. This pattern of cell loss in normal males and females resembles that described by Breedlove (*10*). By P10, the number of SNB motoneurons in males and females had reached its adult level.

To determine if group differences in SNB motoneuron loss correspond to group differences in cell death within the SNB region, we determined the extent and duration of cell death in the SNB. Degenerating cells were counted within the SNB region for all groups at E22, P4, and P10 (*14*), days that span the period of SNB motoneuron loss. Degenerating cells were recognized by their characteristic darkly staining aggregations of chromatin in pycnotic, often fragmented, nuclei and their pale or absent cytoplasm (Fig. 2). Counts of degenerating cells are a sensitive predictor of eventual neuron number (*15*), and they reliably indicate the timing, magnitude, and regional distribution of cell loss (*16*).

During the period of SNB motoneuron loss, when sex differences principally develop, the incidence of degenerating cells was sexually dimorphic and influenced by androgens. At E22, P4, and P10, females had significantly more degenerating cells in the SNB region than males [F(2, 37) = 3.87, P < 0.05], but females treated with TP perinatally did not differ significantly from males (Fig. 3). By P10, at which time SNB motoneuron number had reached its adult level, the incidence of degenerating cells was low for all groups.

These results support the hypothesis that sex differences in SNB motoneuron number emerge largely through the androgenic regulation of normally occurring motoneuron death. Cell death in the SNB occurs at the time when androgenic manipulations permanently determine the number of SNB motoneurons present in adulthood (*7*). Moreover, during the early postnatal period, as sex differences in motoneuron number become prominent, the incidence of cell death in the SNB region is nearly twice as great in females as in males. Finally, and most importantly, perinatal androgen treatment attenuates this cell death in females, reducing SNB motoneuron loss to levels typical of males.

Our results do not exclude the possibility that androgenic influences on the migration or differentiation of SNB motoneurons may also contribute to the development of sex differences (*17*). The prenatal increase in SNB motoneuron number implies that during this period either cells in the SNB region are differentiating into motoneurons or motoneurons are migrating into the SNB region. We favor the latter hypothesis for two reasons. (i) Other lumbar motoneurons with the same mitotic history as the SNB (*8*) are well differentiated at this age, and it would be surprising if SNB motoneurons were cytologically unrecognizable at a time when their cohorts are well-differentiated motoneurons. (ii) Injections of horseradish peroxidase into the LA-BC complex between E18 and P1 label motoneurons not only in the SNB region but also in the area between the SNB and the lateral motor columns. These ectopic cells are typically elongate, with leading and trailing processes, and are not seen after HRP injections into the LA-BC complex after P1 (*18*). We believe these cells reflect a lateral-to-medial migration of SNB motoneurons, producing the prenatal increase in SNB motoneuron number. This increase is sexually dimorphic and influenced by androgen treatment (Fig. 1). Since degenerating cells are present in the SNB region throughout this period, however, androgens could influence the pattern of SNB development prenatally by regulating cell death, migration, or both.

Some pycnotic cells observed in the SNB region may have been degenerating glia or interneurons rather than motoneurons. However, both the time course and extent of motoneuron loss in males and females related to the pattern and incidence of degenerating cells. Moreover, androgen manipulations that prevent SNB motoneuron loss in females correspondingly alter the incidence of

degenerating cells. Because group differences in the incidence of degenerating cells so accurately predict group differences in the relative magnitude of SNB motoneuron loss, we believe that these degenerating cells reflect the death of SNB motoneurons.

Normally occurring motoneuron death is a common feature of the developing spinal cord, and the extent of this death is influenced by interactions between motoneurons and their target muscles (*19*). Androgens promote the survival of both SNB motoneurons and their target musculature, the LA-BC complex. It is possible that androgens act directly on SNB motoneurons to promote their survival, indirectly preventing the involution of the LA-BC complex, since normal muscle differentiation and survival require proper innervation (*20*). Although SNB motoneurons in females do send axons to the periphery before the period of SNB motoneuron death, it is not known if these axons form functional synapses with LA-BC muscle fibers (*18*). Another possibility is that androgens prevent SNB motoneuron death indirectly, by ensuring the survival of their target musculature. However, certain androgenic manipulations can prevent LA-BC involution in females without resulting in male-typical SNB motoneuron numbers (*21*), suggesting that regulation of the number of motoneurons in the SNB region does not depend simply on muscle survival.

It seems likely that androgens prevent the death of SNB motoneurons by interacting with factors normally important for motoneuronal survival. For example, androgens might alter the growth of motoneuronal axons or render axons better able to synapse with their target musculature. Androgens may also regulate the availability of postsynaptic target sites, neurotrophic factors secreted by muscle, or synaptic activity. Establishing which cellular mechanisms are regulated by androgens in this simple neuromuscular system will further our understanding of how these mechanisms contribute to the regulation of cell death throughout the developing nervous system.

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Physiological Effects of Transforming Growth Factor in the Newborn Mouse

Abstract. *Transforming growth factor–type α accelerated incisor eruption and eyelid opening in the newborn mouse and also retarded the growth rates of hair and body weight when administered in high dosage (0.7 to 4 micrograms per gram of body weight). The results of whole animal studies indicate that transforming growth factor–type α and epidermal growth factor do not differ significantly in these effects and suggest that transforming growth factor–type α may be important in immature animals.*

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Transforming growth factor–type α (TGF-α)—also called type I (*1, 2*)—was first isolated from media conditioned by virally transformed cells. It is a mitogenic peptide containing 50 amino acid residues and is operationally defined by its ability to confer reversible phenotypic transformation on NRK fibroblasts (*3*). However, this transforming activity of TGF-α is modulated by another growth factor, transforming growth factor–type β (*4*). Present together, these two transforming growth factors provide for maximal phenotypic transformation. TGF-α displays 32 percent sequence homology with mouse epidermal growth factor (mEGF) (*5*) and exhibits similar activities in competition for binding to the EGF receptor, stimulation of DNA synthesis, and cell growth.

Table 1. Effect of synthetic TGF-α on eyelid opening and incisor eruption in the newborn mouse. Daily subcutaneous injections were made into newborn mice (NCS strain) with 20 μl per gram of body weight. The injections were started with the day of birth (day 0) and given at 24-hour intervals. Daily dosage varied according to body weight, and dosage ranged from 0.03 to 4 μg per gram of body weight. Two animals received injections at each dosage. Control animals received either no injection or dilute phosphate-buffered saline solution. The effects of doses greater than 0.3 μg of both TGF-α and EGF are significantly different from that of the controls (rank sum test, $P < 0.0001$).

Dose (μg/g per day)	Number of animals	Day of	
		Incisor eruption	Eyelid opening
	Control		
No injection	10	9–11	12–14
Saline	14	9–11	12–14
	EGF		
2.7–4	4	6–7	7–8
	TGF-α		
0.03–0.3*	20	9–10	12–13
0.35–0.65†	8	7–8	9–11
0.7–1.4‡	6	6–7	8–10
2.7–4	4	6–7	6–7

* Dose: multiples of 0.03 μg (0.03, 0.06, 0.09, ...), with two mice given each dose. † Dose: 0.35, 0.45, 0.55, and 0.65 μg. ‡ Dose: 0.7, 1.05, and 1.4 μg.