sponse to higher water velocity per se, the flume's pump and filter system were used to vary the concentration of suspended particulate matter (Fig. 3). By the time approximately 40 percent of the particulates (by weight) was filtered out, most worms had withdrawn their tentacles into their tubes. When the experiment was repeated with the filter element removed from the recirculation system, the concentration of suspended particulate matter (and hence the particulate flux) remained constant, as did the number of worms actively feeding. Thus it was not the operation of the pump and filter that inhibited feeding behavior, but the reduced particulate flux.

It has been known for some time that certain benthic organisms, including some spionids, nereids, fabricine sabellids, and oweniids among the polychaetes (*6*), tellinids among the bivalves (*16*), and amphipods among the crustaceans (*17*) are capable of both suspension and deposit feeding. The growing understanding of benthic boundary layer dynamics and rapidly improving flow measurement technology now permit an analysis of the factors influencing the preferred feeding mode. We suggest that animals capable of switching their feeding behavior typify environments of rapidly varying flow characteristics. Rather than being a troublesome anomaly in trophic classifications (*6*), such animals form a distinctive indicator group of their own.

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Hormone Accumulation in a Sexually Dimorphic Motor Nucleus of the Rat Spinal Cord

Abstract. *The fifth and sixth lumbar segments of the rat spinal cord were found to contain a sexually dimorphic nucleus, the spinal nucleus of the bulbocavernosus (SNB). The SNB, which contains motoneurons innervating perineal striated muscles in normal male rats, is diminished or absent in normal females and in males with a genetic mutation rendering them insensitive to androgens. The presence of the nucleus is apparently not dependent on genetic sex, but on the action of androgens. The motoneurons of the adult male SNB accumulate hormone after systemic injections of radioactive testosterone or dihydrotestosterone, but not estradiol, and the SNB motoneurons accumulate more of the injected androgens than do other motoneurons in the same spinal segments. These results demonstrate a morphological sex difference in hormone-sensitive motoneurons that are probably involved in the sexually dimorphic copulatory behavior of the rat.*

Because of the obvious sex differences in the behavior of vertebrates, one might expect to find differences in the central nervous system (CNS) of the two sexes. Raisman and Field (*1*) reported that in rats the "strial portion" of the preoptic area (POA) has more synapses from nonamygdaloid sources in females than in males. Other anatomical sex differences described in the nervous system include the number of autonomic preganglionic neurons in cats, and the dendritic field patterns in the POA of hamsters (*2*). Nottebohm and Arnold (*3*) reported gross sexual dimorphism in the size of certain brain nuclei of song birds. These nuclei are larger in males and are known to play a role in the singing behavior which only males display. Gorski *et al.* (*4*) found a strikingly dimorphic nucleus in the rat POA which is larger in males than in females. Because the last two dimorphisms are easily detected, they provide convenient measures of the process of sexual differentiation of the brain, and therefore study of these systems may lead to a better understanding of the factors critical to sexual differentiation of vertebrate behavior. We now report a prominent dimorphism in a motor nucleus of the rat spinal cord, the neurons of which accumulate radioactivity after injections of tritiated androgens but not estradiol. This dimorphism is useful for studying sexual differentiation of the CNS because it offers advantages distinctive to motoneurons, for example, readily determined behavioral function, electrophysiological accessibility due to the large somas, relatively simple inputs and outputs, and ease of study in early development.

Motoneurons were identified by injecting horseradish peroxidase (HRP) into each of the three striated muscles attached to the rat penis: the ischiocavernosus (IC), bulbocavernosus (BC), and levator ani (LA) (*5*). The muscles of 30 male anesthetized Sprague-Dawley rats were injected with 3 to 50 μl of a 30 percent solution of HRP (Sigma type VI) in saline by means of a 10-μl syringe under a dissecting microscope (*6*). Multiple injections were used to distribute the HRP evenly throughout the muscle, and afterward the exposed perineal region was flushed with saline (*7*). The animals were killed 24 hours later and the retrogradely transported HRP was stained in the spinal cord segments caudal to thoracic 13 (*8*).

After the injection of HRP into IC, retrogradely labeled motoneurons were found in the extreme ventrolateral quadrant of the ventral horn in the fifth and sixth lumbar spinal segments (L_5 and L_6). Injection of HRP into either the BC or LA resulted in labeled cells being found in the dorsomedial portion of the ventral horn, 50 to 250 μm from the midline and 200 to 400 μm below the ventral margin of the central canal (Fig. 1a, ar-

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row). These latter neurons form a compact nucleus extending 1.5 mm from caudal L_5 to rostal L_6, on the border of the ventral funiculus of white matter. The neurons of this nucleus are large (30 to 50 μm in diameter), multipolar, and stain densely for Nissl substance. Because we believe this nucleus is hitherto undescribed, we propose naming it the spinal nucleus of the bulbocavernosus (SNB). This name seems appropriate since other evidence suggests that the LA may more properly be called the dorsal bulbocavernosus (*9*). Comparison of thionin-stained L_5 and L_6 cord sections from male and female rats revealed that in females there are fewer cells in the region (*10*) and the few cells present are smaller than those of the male SNB, resulting in the apparent absence of the SNB in female rats (Fig. 1, a and b).

Because some motoneurons of the male rat spinal cord accumulate radioactivity after injection of tritiated dihydrotestosterone (DHT) (*11*), and because the position of cells in the SNB make them readily distinguishable, we used autoradiography to determine whether these particular motoneurons accumulate hormones. Adult male rats were castrated and adrenalectomized, then maintained on drinking water containing 0.9 percent saline. Two days later an intra-atrial catheter was implanted, and 24 hours after catheter implantation the animals were injected with tritiated DHT, testosterone (T), or estradiol (E) (1.2 nmole per 100 g of body weight in a 0.3-ml vehicle of 50 percent ethanol) (*12*). One hour after injection the rats were decapitated and the lumbar and sacral sections of the spinal cord were removed. Each cord was cut into blocks two spinal segments long and frozen with dry ice. The tissue was cut in 6-μm transverse sections in a cryostat at −20°C, and placed on microscope slides previously coated with nuclear track emulsion. The autoradiograms were photographically developed 29 to 258 days later, counterstained with thionin, and examined under a microscope (*13*).

Table 1. Percentage of SNB or VLMN cells that are labeled after injection of tritiated hormone, according to either the Poisson or five-times-background criteria.

Hormone	SNB		VLMN	
	Poisson	5×	Poisson	5×
DHT	97.6	68.6	96.4	34.9
T	96.0	40.1	77.4	7.7
E	3.3	0	5.8	0

To decide whether a given cell was significantly labeled, we used a Poisson model of the distribution of reduced silver grains. The expected number of grains over the nucleus of a cell was calculated from the density of grains over the background (adjacent unstained neuropil) and the area of the cell's nucleus. This expected number was then used as the mean of a Poisson distribution describing the number of grains that would occur over that cell's nucleus by chance. If the actual number of silver grains over the nucleus was more than would be expected by chance for that Poisson distribution ($P < .01$), then the cell was considered labeled. This criterion is less stringent than the more frequently used criterion of five times background density (*14*), but the Poisson distribution provides a more accurate evaluation of whether a cell is labeled. Each hormone was injected into three males and at least

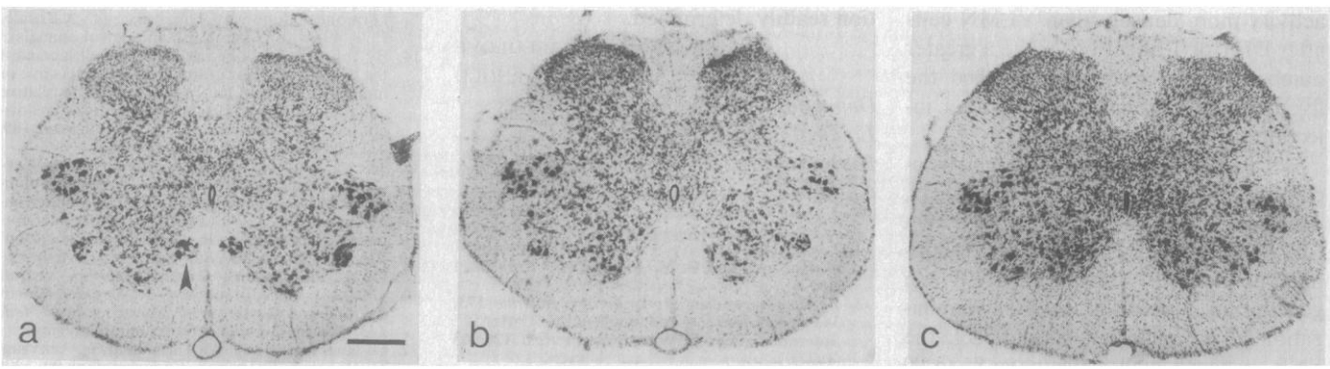

Fig. 1. Sections from the lumbar segments of the rat spinal cord. (a) and (b) Thionin-stained 50-μm transverse sections from male and female fifth lumbar segments, respectively. The arrow in (a) points to the left spinal nucleus of the bulbocavernosus (SNB) of the male. Note the virtual absence of the SNB in the female cord in (b). Scale bar, 400 μm. (c) Section of the lumbar spinal cord of a genetically male rat which because of the testicular feminization (*Tfm*) mutation, possesses few androgen receptors. Despite the fact that this is a genetic male, the SNB is absent, implying that the interaction of androgens with their receptors is important to the development of the SNB. Normal male littermates of the *Tfm* males have a normal SNB. Magnification as in (a) and (b).

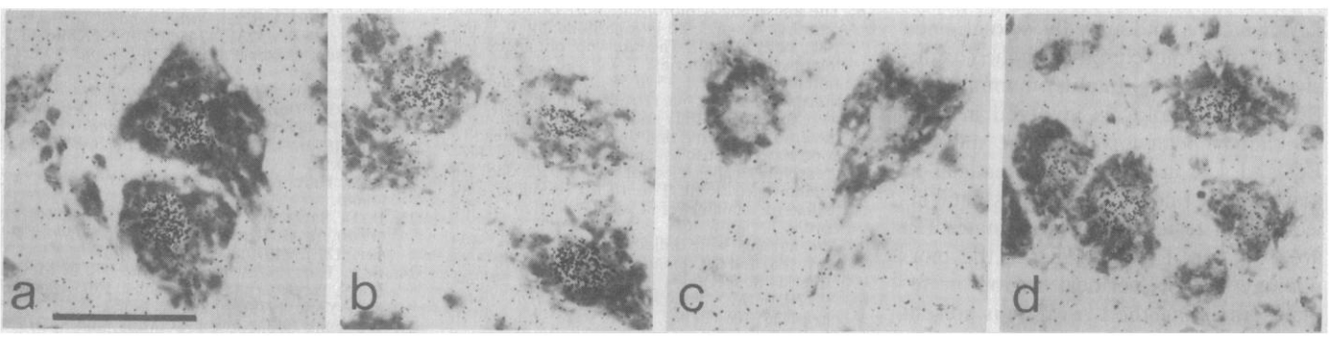

Fig. 2. Autoradiograms from the fifth and sixth lumbar segments of the male rat spinal cord, stained with thionin (scale bar, 50 μm). (a to c) Cells of the spinal nucleus of the bulbocavernosus (SNB) after injections of tritiated DHT, T, and E, respectively. Note accumulation of hormone over the relatively unstained nucleus in (a) and (b), but not in (c). (d) Ventrolateral motoneurons of the fifth lumbar segment after injection of tritiated DHT. Exposure periods: (a) 211, (b) 67, (c) 64, and (d) 211 days.

50 SNB cells were analyzed from each. The SNB neurons were easily identified by location, large somas, multipolar shape, and dense staining. For purposes of comparison, the nuclei of 50 large, multipolar, densely staining motoneurons in the ventrolateral portion of the L_5 and L_6 cord sections were examined. This population includes but is not exclusively the motoneurons of IC. The observer analyzing the autoradiograms was unaware of which hormone had been injected (Fig. 2).

The percentages of SNB cells labeled by the three hormones are shown in Table 1, where both the Poisson and the five-times-background criteria are listed. With either criterion, more SNB cells are labeled after DHT or T injections than E (*15*). Injection of E resulted in densely labeled cells being found in the dorsal horn and lamina X (*16*). The SNB neurons accumulated hormone more heavily after DHT than T injections, since a greater proportion of SNB cells reached the more stringent five-times-background criterion after DHT injection (*15*). More of the SNB cells accumulated T or its metabolites than did the ventrolateral motoneurons (VLMN) (Table 1). The SNB cells also accumulated radioactivity more densely than VLMN cells after DHT or T injections since a greater number of the SNB cells reached the five-times-background criterion after injection of these hormones (*15*).

Because the SNB cells of adult rats accumulate androgens but not estrogens, one might expect that androgens play a role in the sexually dimorphic development of the SNB. Thus, King-Holtzman genetic males with the testicular feminization (*Tfm*) mutation should lack the SNB, because such males have 85 to 90 percent fewer androgen receptors (*17*). Examination of the spinal cords of *Tfm* males confirmed the predicted absence of an SNB in these animals (Fig. 1c) (*18*).

The function of the SNB may be inferred from the function of the BC and LA, which is undoubtedly sexual since both of these muscles are attached exclusively to the penis. In other mammals the BC or its homolog is involved in male copulatory behavior (*19*). The muscles BC and LA are absent or vestigial in adult female rats (*9*). Pre- or postnatal injections of testosterone propionate (TP) are effective in masculinizing the morphology of the perineal region in females, including the LA which is present in female rats at birth, but atrophies in the first 3 weeks of life unless maintained by postnatal injections of TP (*20*). Such perinatal androgen injections in females also masculinize their copulatory behavior (*21*). Therefore, the finding that the neurons of the SNB accumulate androgens or their metabolites, but not E, together with the neonatal androgen sensitivity of the SNB's target muscles, suggest that androgens but not estrogens play a role in the development of the dimorphism of the SNB. This hypothesis is supported by the absence of the SNB in *Tfm* males with reduced androgen receptors.

The penile striated muscles are involved in reflexes discovered by Hart (*22*), which are controlled by the spinal cord, and these reflexes may be related to copulatory behavior (*23*). Androgens, acting in the spinal cord, augment these reflexes, but E does not. Neonatal castration of males permanently reduces the frequency of such reflexes (*24*). The accumulation of hormone by the SNB motoneurons after androgen but not E injection suggests that the SNB is a site of action of androgens in modifying the penile reflexes. If this were the case, then the SNB cells provide significant advantages for studying the mechanisms by which a hormone modifies behavior of a vertebrate, because these neurons are large, well localized, and their behavioral function readily determined.

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6. The anesthetic (Chloropent; Fort Dodge Laboratories) was injected intraperitoneally (0.33 ml/100 g body weight). The rats were obtained from Simonsen Laboratories.
7. Initially large (total, 15 to 50 μl) unilateral injections were made, but retrogradely labeled cells were found bilaterally and in several locations in the cord, indicating that the HRP had spread to contralateral muscles and nearby tissue. Progressively smaller injections were made until the volume used (total, 3 to 10 μl) produced only ipsilateral staining of a single portion of the cord.
8. The animals were killed with Chloropent and perfused, and the spinal cords were stained as in either J. S. DeOlmos [*Exp. Brain Res.* **29**, 541 (1977)] or M. Mesulam [*J. Histochem. Cytochem.* **26**, 106 (1978)]. Cords were sectioned in 50-μm transverse or horizontal sections.
9. K. J. Hayes [*Acta Endocrinol.* **48**, 337 (1965)] states that the LA muscle of the rat is misnamed because (i) LA is not the first name applied to this muscle, (ii) the attachments of the rat LA are not homologous to the LA of humans, and (iii) the muscle could not possibly levate the anus. Hayes suggests that the first application of the name LA to this muscle by Greene [see (*5*)] may have been an error, and proposes the muscle be called the dorsal bulbocavernosus. Our finding a common locus of LA and BC motoneurons lends weight to Hayes' suggestion. Other authors disagree with the newly proposed name, but agree that the term LA is erroneously applied to this muscle in the rat (*20*).
10. As part of another experiment (S. M. Breedlove and A. P. Arnold, in preparation) adult Sprague-Dawley male and female rats were sham castrated and injected with sesame oil vehicle for 28 days. Then the rats were killed with an overdose of Nembutal and perfused intracardially with saline, then buffered Formalin. The spinal cord was removed with the dorsal roots being used for a guide, and alternate transverse 50-μm sections were stained with thionin. The number of nuclei of densely stained cells in alternate sections in the region described (200 to 400 μm ventral to the central canal and within 250 μm of the midline in L_5 and L_6) counted by a blind observer was: males (N = 4) 172.3 ± 11.59 [standard error of mean (S.E.M.)]; females (N = 5) 46.9 ± 11.17. These numbers are corrected for split nuclei by the method of M. Abercrombie [*Anat. Rec.* **94**, 239 (1946)].
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13. Kodak NTB3 emulsion was used. The autoradiographic method was a modification of that used by Pfaff and Keiner (*16*). For each animal, one-third of the autoradiograms was developed after one of three different intervals to provide a range of background silver densities. The mean (± standard deviation) background densities for the three hormones were: DHT, 6.76 (± 3.0); T, 12.22 (± 3.09); E, 10.98 (± 8.31) silver grains per 100 μm^2. There were no significant differences in the background density of the three hormones (one-way analysis of variance, $P > .40$, with N being the number of animals).
14. By this criterion one considers a cell labeled if the density of silver grains over the cell is five or more times that of the background. The relative advantages of various labeling criteria are discussed in A. P. Arnold [*J. Comp. Neurol.* **189**, 421 (1980)].
15. The P value, $< .05$, was obtained by the two-tailed, independent t-test, with N being the number of animals and the test being done after angular transformation of the data to obtain a normal distribution [R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969)].
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