of 6- to 8-nm thick filaments (Fig. 4, b and c). Individual filaments had substructural periodicity similar to that of actin filaments (*15*). The estimated number of filaments per cable varied from 10 to more than 80 (*16*).

Since actin filaments were the only detectable linear components of the cables, it is likely that single actin filaments are the structural units of the multiple tracks at the surface of the cables. The requirement of ATP to support organelle movement along the actin filaments suggests that an actin-activated adenosinetriphosphatase was directly involved in this process. Since movement did not require Ca^{2+}, it may have been independent of regulatory proteins associated with the actin filament such as troponin and tropomyosin (*17*). Perhaps only actin and an actin-activated adenosinetriphosphatase present at the surface of the organelle are involved in movement in this system. Evidence from several physiological and pharmacological experiments supports the contention that myosin or a myosin-like adenosinetriphosphatase is responsible for the generation of the motive force (*2, 18*). Further electron microscopy is needed to ascertain whether small cross-bridging structures, similar to the myosin arms in muscle (*19*), are involved in the interactions between organelles and actin filaments.

The observation that a single transport substrate could support movement of different organelles at different rates indicates that only the part of the motile machinery that is associated with an organelle may modulate its rate of movement (*20*). Although this may be the case in other systems where a variety of patterns of organelle movement has been observed (*1, 21*), microtubules appear to be the basic substrate for organelle motility in axons and cultured cells (*21*).

The preparation described here permits extensive analysis of cytoplasmic organelle movement in the absence of an organized cytoplasm and without the insulating effect of a plasma membrane. It permits direct visualization and measurement of organelle displacements along the transport substrate. This preparation is easy to obtain, highly reproducible, and amenable to biochemical, pharmacological, and mechanical manipulations.

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References and Notes

1. L. T. Rebhun, *Int. Rev. Cytol.* **32**, 93 (1972); R. Goldman, T. Pollard, J. Rosenbaum, Eds., *Cell Motility* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1976), p. 1373; B. Grafstein and D. S. Forman, *Physiol. Rev.* **60**, 1168 (1980); M. Schliwa, in *Cell and Muscle Motility*, J. W. Shaw, Ed. (Plenum, New York, 1984), vol. 5, p. 1.
2. N. Kamiya, *Annu. Rev. Plant Physiol.* **32**, 205 (1981).
3. D. B. Sattelle and P. B. Buchan, *J. Cell Sci.* **22**, 633 (1976); E. A. Nothnagel and W. W. Webb, *J. Cell Biol.* **94**, 444 (1982).
4. R. Jarosh, *Phyton (Horn, Austria)* **6**, 87 (1956); R. E. Williamson, *J. Cell Sci.* **10**, 811 (1972); T. Hayama and M. Tazawa, *Protoplasma* **102**, 1 (1980); S. Higashi-Fujime, *J. Cell Biol.* **87**, 569 (1980).
5. S. Inoue, *J. Cell Biol.* **89**, 346 (1981); R. D. Allen and N. S. Allen, *J. Microsc.* **129**, 3 (1983). The video camera used was a Dage-68 (Dage-MTI, Michigan, Ind.) and the computer frame memory for background subtraction was IVS-I (IVS, Boston, Mass.).
6. R. Nagai and L. I. Rebhun, *J. Ultrastruct. Res.* **14**, 571 (1966); E. Kamitsubo, *Protoplasma* **74**, 53 (1972); N. S. Allen, *J. Cell Biol.* **63**, 270 (1974).
7. Structures with dimensions equal to or smaller than the resolution limit of the light microscope (0.1 to 0.2 μ) appear inflated by diffraction. It is possible to visualize structures as small as 50 nm with the video contrast enhancement (*5*); however, it is not possible to correlate the apparent diameter seen on the video images with the real diameter of the structures.
8. Velocity was determined directly from the television screen by measuring the time required for an organelle to travel 5- to 25-μm distances. The data were derived from 100 values and given as the mean ± standard error of the mean.
9. V. R. Franceschi and W. J. Lucas, *J. Ultrastruct. Res.* **75**, 218 (1981).
10. Because of the low frequency and variable shape of the fast moving organelles, most of the analysis of the movements described was derived from the slow-moving organelles that had a clearly defined spherical shape.
11. An average velocity of 1.6 ± 0.1 μm/sec was observed for the spherical organelles in an 18-hour-old preparation.
12. R. S. Adelstein and E. Eisenberg, *Annu. Rev. Biochem.* **49**, 921 (1980).
13. L. S. Barak *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 980 (1980).
14. J. E. Heuser *et al.*, *J. Cell Biol.* **81**, 275 (1979).
15. N. Hirokawa and J. E. Heuser, *ibid.* **91**, 399 (1981).
16. These filaments were identified as F actin through the formation of arrowhead structures upon labeling with heavy meromyosin [R. E. Williamson, *Nature (London)* **248**, 801 (1974); B. A. Palevitz and P. K. Hepler, *J. Cell Biol.* **65**, 29 (1975)]. These arrowheads point upstream in relation to the cytoplasmic streaming in intact cells [Y. M. Kersey, P. K. Hepler, B. A. Palevitz, N. K. Wessells, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 165 (1976)].
17. R. D. Valle *et al.*, *J. Cell Biol.*, in press.
18. T. Kato and Y. Tonomura, *J. Biochem. (Tokyo)* **82**, 777 (1977); R. E. Williamson, *Eur. J. Cell Biol.* **20**, 177 (1979); M. P. Sheetz and J. A. Spudich, *Nature (London)* **303**, 31 (1983) described directional movement of heavy meromyosin–coated fluorescent beads when placed on the surface of a preparation of cortical components of the cytoplasm of *Nitella*. However, the rate of movement of the coated beads was an order of magnitude slower than the movement of the endogenous organelles.
19. H. E. Huxley, *Nature (London)* **243**, 445 (1973).
20. An important question concerns the manner in which two distinct velocities are produced along the same actin substrate if we assume that the basic mechanism is the same in both organelle types. It is possible that there are two adenosinetriphosphatases with different activities or that there is a configurational difference in the arrangement of an adenosinetriphosphatase at the surface of each organelle type.
21. J. H. Hayden, R. D. Allen, R. D. Goldman, *Cell Motility* **3**, 1 (1983); B. Kachar, P. C. Bridgman, H. Arnheiter, *J. Cell Biol.* **99**, 49a (1984); B. Kachar, P. C. Bridgman, T. S. Reese, *ibid.*, p. 50a; Schnapp *et al.*, *Anat. Rec.* **208**, 157A (1984).
22. I thank Dr. T. S. Reese for continuous support, Dr. P. Bridgman for assistance with the "fast-freezing," and J. Fex, R. Wagner, and M. Brightman for valuable discussions and revision of the manuscript. I dedicate this report to my parents, Claire and Mikhael Kachar.

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Hormonal Control of the Anatomical Specificity of Motoneuron-to-Muscle Innervation in Rats

Abstract. *Motoneurons of the spinal nucleus of the bulbocavernosus innervate bulbocavernosus muscles in male rats. Adult female rats normally lack both the spinal nucleus and its target muscles. Prenatal treatment of females with testosterone propionate resulted in adults having, like males, both the spinal nucleus and its target muscles. However, prenatal treatment with dihydrotestosterone propionate preserves the muscles but not the motoneurons. This paradoxical condition might result from (i) bulbocavernosus muscles without innervation; (ii) muscles innervated by morphologically unrecognizable motoneurons; (iii) muscles innervated by a very few spinal nucleus cells, each innervating many bulbocavernosus fibers; or (iv) muscles innervated by motoneurons outside their normal anatomical locus in the spinal nucleus. The results of retrograde marker injections into the bulbocavernosus muscles of females treated with androgen refute the first three possibilities and confirm the last: the different androgen treatments result in anatomically distinct spinal motor nuclei innervating bulbocavernosus muscles.*

The anatomical specificity of neural connections is undoubtedly crucial to behavioral function, and the achievement of this specificity poses one of the fundamental questions for developmental neurobiology. Most studies addressing this question monitor the neural connectivity in surgically manipulated individuals and then make inferences about the mechanisms guiding the formation of connections in normal subjects. Another approach is to study neural systems that differ anatomically between the two sexes. These sexually dimorphic neural systems can follow either of two developmental programs: that which results in the masculine anatomical configuration, or that which produces the feminine con-

figuration. An advantage of this approach is that the experimenter can manipulate development by altering the cues (steroid hormones) that normally determine the program to be followed.

One such sexually dimorphic system is the spinal nucleus of the bulbocavernosus (SNB), which consists of motoneurons innervating the perineal bulbocavernosus (BC) muscles in male rats (*1*). While these striated muscles are crucial for reproduction in male rats (*2*), adult females normally lack the BC muscles and have so few motoneurons in the SNB region that no coherent nucleus is formed. The BC muscles and SNB are present in female rats at birth, but the muscles disappear and most of the SNB cells die shortly thereafter (*3*). Treatment of females just after birth with either testosterone propionate (TP) or dihydrotestosterone propionate (DHTP) prevents this dissolution and results in adults which, like males, have an SNB and the striated BC muscles. However, prenatal administration of the two androgens exerts disparate effects on the SNB system. Prenatal treatment of females with TP causes the muscles and the SNB to persist into adulthood. Prenatal DHTP treatment also causes the BC muscles to persist, but these animals have no more motoneurons in the SNB region than normal females, which lack the target muscles altogether (*4*). This paradoxical condition of females treated with DHTP might result from (i) BC muscles without innervation, (ii) muscles innervated by morphologically unrecognizable motoneurons, (iii) muscles innervated by a very few SNB cells, each innervating many BC fibers, or (iv) BC muscles innervated by motoneurons outside their normal anatomical locus in the SNB. In these experiments, I injected horseradish peroxidase (HRP) into the BC muscles of females treated with androgen to demonstrate that the last possibility is correct: the different androgen treatments resulted in anatomically different spinal motor columns innervating BC muscles.

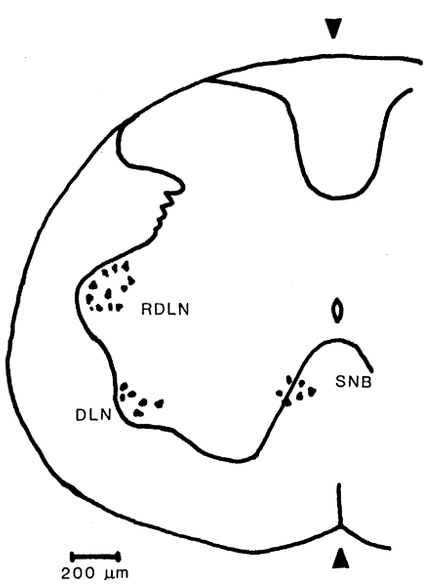

Fig. 1. Schematic drawing of a transverse section of the rat spinal cord showing the location of motoneuronal nuclei in the fifth and sixth lumbar segments. Both male and female rats have a dorsolateral nucleus (DLN) and a retrodorsolateral nucleus (RDLN). Adult female rats do not normally have the perineal bulbocavernosus (BC) muscles and consequently have few motoneurons in the region of the spinal nucleus of the bulbocavernosus (SNB), which innervates the BC in males. Prenatal treatment of female rats with either of two androgens, testosterone propionate (TP) or dihydrotestosterone propionate (DHTP) will result in BC muscles in adulthood. Only the prenatal TP results in more motoneurons in the medial SNB region. Arrows indicate the midline; scale bar, 200 μm.

On each of gestational days 17 to 22, pregnant Sprague-Dawley rats (Simonsen) were injected subcutaneously with 2 mg of either TP or DHTP (Steraloids). Upon delivery (day 23), female pups were toe-marked and cross-fostered to untreated dams that had delivered the same day. Pups were weaned at 21 days of age and as adults received testosterone implants (*5*) to increase the size of the BC muscles and thereby facilitate later injections. At least 2 weeks after the capsule was implanted, the treated females were anesthetized (Chloropent, 3 ml/kg, intraperitoneally), and the lateral-ventral BC muscles on each side were injected with 0.5 to 1.0 μl of 30 percent HRP (Sigma type VI) in saline delivered through a microsyringe guided under a dissection microscope (*6*). The rats were killed with a barbiturate overdose 24 to 48 hours later and the spinal cords were horizontally sectioned and stained for HRP by a tetra-methylbenzidine method (*7*). Most of the tissue was examined without counterstaining to maximize the detection of HRP-filled motoneurons. Because the aim of the experiment was to compare TP and DHTP females, the animals were treated as seven yoked pairs, each consisting of one DHTP and one TP female. Each pair of animals was treated together, through suckling, implanting with testosterone capsules, injection with HRP, being killed, perfused, sectioned, and stained together. The HRP-stained sections from all treated females, as well as from seven control males (injected with 3 to 6 μl of HRP into the BC on each side) were analyzed together by an observer who was unaware of the treatment conditions.

Of the four possible explanations for the paradoxical effects of prenatal DHTP, HRP results rule out the first three and conform to the last. HRP injections into the BC of DHTP-treated females retrogradely fill ventral horn cells, which in Nissl counterstain reveal all the morphological signatures of motoneurons; that is, they are large, multipolar, and densely Nissl-staining. Because the BC is innervated by morphologically normal motoneurons, the first two possibilities are excluded. If the third possibility were correct—if the BC of DHTP-treated females were innervated by the very few remnant SNB motoneurons, each innervating a large number of fibers—one would expect a given volume of HRP injected into the BC to fill fewer motoneurons in the DHTP females than in the TP females. However, there was no significant difference in the number of motoneurons filled by HRP injections in the two groups [TP females: 42.3 ± 9.7 (standard error of the mean) filled cells per microliter of HRP solution injected;

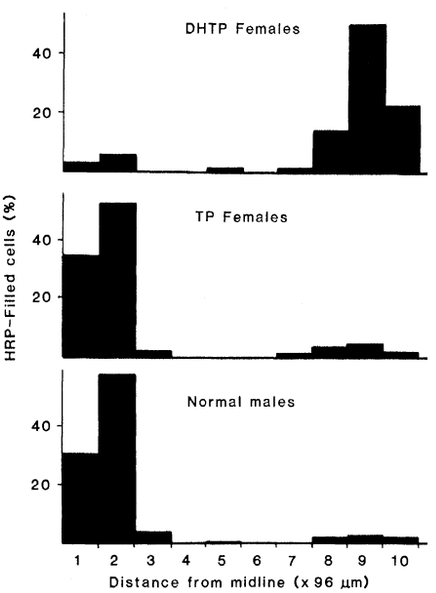

Fig. 2. The medial-lateral position of motoneurons innervating the lateral-ventral bulbocavernosus (BC) muscle revealed by retrograde HRP labeling. In normal males most motoneurons innervating the BC are within 288 μm of the midline, within the boundaries of the SNB. Female rats given TP before birth (544 HRP-filled cells) show an identical distribution of motoneurons innervating the BC, but females prenatally treated with DHTP show a different arrangement (representing 489 HRP-filled cells). In these females, the motoneurons innervating the BC are in a lateral column of the spinal cord, specifically in the dorsolateral nucleus (Fig. 1). The number of HRP-filled motoneurons per volume of injected HRP did not differ in the two groups of females. This lateral position of BC motoneurons in DHTP-treated females explains the absence of motoneurons in the SNB region of such females, despite the presence of BC muscles.

DHTP: 39.8 ± 8.0 filled cells; $P > 0.40$, matched-pairs t-test]. The ratio of motoneurons innervating muscle fibers is similar in the two groups and cannot explain the unusual condition of the DHTP-treated females.

On one feature, the anatomical location of the HRP-filled motoneurons, DHTP and TP females were drastically different. In adult females given TP prenatally, most motoneurons innervating the BC were found in the same location as in males: the medial motoneuronal column normally occupied by the SNB (Figs. 1 and 2). However, the BC of females treated with DHTP was innervated by motoneurons in an anomalous, lateral location—the DLN motoneuronal column 500 μm away (Fig. 2). HRP injections into male BC virtually never labeled lateral motoneurons (Fig. 2). Thus, TP produces females with BC muscles primarily innervated by the same motoneuron column as in males, the medial SNB pool. But DHTP produces females with muscles innervated by an anatomically different and, relative to males, abnormal motoneuronal pool.

Vertebrate motoneurons innervating a given muscle are organized in rostrocaudally oriented columns occupying the same spinal location with consistency across individuals (*8*), making the neuromuscular system a valuable model of the specificity of connections in the nervous system (*9*). However, to my knowledge, no one has completely accounted for the development of this specificity. These results represent an example of the effect of manipulation of an identified, naturally occurring developmental cue (androgen) on the pattern of neuromuscular connectivity. Testosterone is the principal androgen produced by rat testes, and dihydrotestosterone is the major active metabolite in the periphery (*10*). The different configurations of innervation produced by the two androgens suggest that the normal pattern of connectivity in the SNB system may be determined by relative contributions of the two hormones, which is determined by the activity of 5α-reductase (E.C.1.3.99.5), the enzyme controlling the irreversible conversion of testosterone to 5α-dihydrotestosterone (*10*). The activity of 5α-reductase varies considerably across rat brain regions (*11*), and there is evidence of variable reductase concentrations in motoneuronal nuclei of the rat lumbar spinal cord (*12*). Development of the normal innervation pattern by the SNB may require the regional inhibition of testosterone's conversion to dihydrotestosterone, which would be circumvented by the direct administration of DHTP. Prenatal DHTP treatment could accomplish this unique innervation pattern by altering the migration, death, or axonal outgrowth of developing motoneurons. By whichever mechanism such disparate results are accomplished, they represent a unique example of alterations in the pattern of motoneuron-to-muscle innervation through the use of normally relevant developmental cues. An understanding of how this anomalous pattern comes about may elucidate mechanisms by which normal innervation patterns are achieved.

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References and Notes

1. S. M. Breedlove and A. P. Arnold, *Science* **210**, 564 (1980). The SNB innervates three bilaterally symmetrical perineal muscles: the medial-ventral BC, the lateral-ventral BC, and the dorsal BC, also known as the levator ani.
2. B. D. Sachs, *J. Reprod. Fertil.* **66**, 433 (1982); B. L. Hart and Y. Melese-d'Hospital, *Physiol. Behav.* **31**, 807 (1983).
3. R. Cihak, E. Gutmann, V. Hanzlikova, *J. Anat.* **106**, 93 (1970); S. M. Breedlove, *Soc. Neurosci. Abstr.* **10**, 927 (1984); E. J. Nordeen, K. W. Nordeen, D. R. Sengelaub, A. P. Arnold, *ibid.*, p. 453.
4. S. M. Breedlove and A. P. Arnold, *J. Neurosci.* **3**, 424 (1983).
5. The capsules were implanted at 54 to 70 days of age and consisted of 3 cm lengths of Silastic tubing filled with crystalline testosterone and sealed by the method of E. R. Smith, D. A. Damassa, and J. M. Davidson [in *Methods in Psychobiology*, R. D. Meyers, Ed. (Academic Press, New York, 1977), vol. 3, p. 259].
6. The BC muscles in females treated with androgen are readily recognized as striated muscle ensheathing the bulbar base of the phallus, which is intermediate in size between a normal clitoris and penis.
7. M. Mesulam, *J. Histochem. Cytochem.* **26**, 106 (1978).
8. G. J. Romanes, *Prog. Brain Res.* **11**, 93 (1964).
9. C. Lance-Jones and L. Landmesser, *J. Physiol. (London)* **302**, 581 (1980); L. Landmesser, *Annu. Rev. Neurosci.* **3**, 279 (1980).
10. L. Martini, *Endocrine Rev.* **3**, 1 (1982).
11. M. K. Selmanoff, L. D. Brodkin, R. I. Weiner, P. K. Siiteri, *Endocrinology* **101**, 841 (1977).
12. Although no rat lumbar motoneurons accumulate estrogen, virtually all of them accumulate radioactivity after the injection of tritiated dihydrotestosterone, indicating that all lumbar motoneurons have androgen receptors. However, after the injection of tritiated testosterone, only some motoneurons (including most SNB cells) densely accumulate steroid [S. M. Breedlove and A. P. Arnold, *J. Comp. Neurol.* **215**, 211 (1983)]. Since all of these motoneurons have androgen receptors, the variable accumulation after testosterone injection suggests that motoneurons vary in the activity of the enzyme 5α-reductase which converts testosterone to dihydrotestosterone.
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Expression of Myelin-Associated Glycoprotein by Small Neurons of the Dorsal Root Ganglion in Chickens

Abstract. *Biochemical and immunocytochemical investigations have shown that myelin-associated glycoprotein (MAG) is exclusively related to myelin and myelin-forming cells in mammals. In the present study it was found that dorsal root ganglia in young chickens display MAG-immunoreactive material in most small sensory neurons. The presence of MAG at the surface of small sensory neurons raises the question of whether this glycoprotein acts as a cell adhesion molecule in lower vertebrates.*

Myelin-associated glycoprotein (MAG), an intrinsic glycoprotein of high molecular weight, is specifically located in myelinating oligodendrocytes but not in neurons, astrocytes, or other cells of the central nervous system in mammals (*1–3*). In rat peripheral nerves antiserum to MAG stains myelinating Schwann cells and the periaxonal regions of myelinated nerve fibers but not trigeminal ganglion cells (*3*). Hence it has been postulated that MAG may play a key role in starting myelinogenesis before compaction of the lamellae (*1–3*).

We used a rabbit antiserum raised against MAG purified from rat myelin (*4*) as an immunocytochemical probe of the dorsal root ganglia (DRG) of 5- to 7-day-old chickens. A first series of experiments was carried out with light microscopic immunocytochemical methods (*5*). Except for the expected reaction in myelinating Schwann cells (Fig. 1A), small ganglion cell bodies located mainly in the mediodorsal region of the DRG displayed the strongest immunostaining (Fig. 1A). Most of the small ganglion cells reacted with antiserum to MAG, whereas the large ganglion cells were free of any staining (Fig. 1B). In the small neurons the immunoprecipitate extended throughout the whole perikaryon, with strong accumulation in cytoplasmic areas suspected to correspond to the Golgi apparatus (Fig. 1B). This location was confirmed by electron microscopy (Fig. 1C) with a preembedding immunocytochemical procedure (*5*). The immunoreactive precipitates were related to cisternal membranes of the rough endoplasmic reticulum and concentrated in the saccules of the Golgi apparatus and in a few vesicles (Fig. 1C). Furthermore, reactive products were located at the cell surface of small ganglion cells (Fig. 1, B and D). The presence of MAG-immuno-