The longitudinal muscles of the chaetognaths have a well-developed sarcoplasmic reticulum (*21*) and are therefore presumably not obligated to use extracellular calcium for contraction, as they have an internal source of the ion. They could use sodium rather than calcium currents to depolarize the muscle and trigger force generation mainly by release of calcium from the sarcoplasmic reticulum. One possible selective pressure that would favor the use of sodium rather than calcium currents would be the faster kinetics afforded by the use of the former, which would in turn allow faster muscle responses. This feature is of obvious value to predatory animals such as chaetognaths (*8, 9*). At this point one can only speculate about the evolution of sodium channels in muscle. It is possible that sodium action potentials are a trait of all deuterostome muscles that have a well developed sarcoplasmic reticulum. Alternatively, sodium channels could have evolved independently in the muscles of both sagitta and the chordates. With the development of the loose patch clamp, it will now be possible to examine the small muscles of other invertebrate phyla and perhaps provide some insight into this question.

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10. *Sagitta elegans* were obtained from plankton tows at a depth of 200 m from Puget Sound and kept at 4°C. *Sagitta* are approximately 1.5 cm long and torpedo shaped, with a diameter of about 1.0 mm. Experiments were performed in artificial seawater (ASW): 460 m*M* NaCl, 6.7 m*M* KCl, 2.5 m*M* $CaCl_2$, 1.3 m*M* $MgSO_4$, 4.4 m*M* Na_2HPO_4, 10 m*M* Hepes buffer, *p*H 7.4. (Sodium-free ASW had the same composition except that tris-chloride replaced NaCl). Unless stated otherwise, all experiments were performed at 4° to 6°C.
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12. For electrophysiological examination, animals were opened mid-dorsally and the viscera were removed to expose the longitudinal muscles running the length of the body. These muscles have 2.4-μm repeating sarcomeres and a diameter of approximately 15 μm. These preparations were then mounted in a plexiglass chamber and flooded with ASW. Resting and action potentials were recorded with intracellular glass microelectrodes filled with 3*M* KCl and having resistances of 7 to 15 megohms. Action potentials from *Sagitta* were elicited with extracellular bipolar platinum electrodes. Action potentials were generated in pharate adult *Manduca* by stimulating the ventral nerve cord with bipolar platinum electrodes while recording from the lateral intersegmental muscle of the fifth abdominal segment. The composition of the saline was 35 m*M* KCl, 9.2 m*M* NaCl, 4.2 m*M* $CaCl_2$, 16 m*M* $MgCl_2$, 172 m*M* glucose, 2.5 m*M* phosphate buffer, *p*H 6.7. The action potential in the frog sartorius muscle was produced by stimulating the muscle directly with bipolar platinum electrodes. The saline consisted of: 115 m*M* NaCl, 2.5 m*M* KCl, 1.8 m*M* $CaCl_2$, 3 m*M* phosphate buffer, *p*H 7.25.
13. Voltage-clamp measurements were performed with the extracellular loose-patch clamp as described elsewhere (*15*). Briefly, glass capillaries with fire-polished tips approximately 10 μm in diameter were filled with saline and pressed against a fiber to electrically isolate a patch of the sarcolemma. Voltage steps were applied to the electrode, and the resulting current flow was measured and corrected for leakage and capacitive artifacts. A holding potential of −20 mV was applied to the patch. In some experiments, muscles were initially treated for 5 minutes at 0°C with 1 mg of type III collagenase (Sigma) (E.C. 3.4.24.3) per milliliter of ASW to disrupt the connective tissue over the muscle to achieve tighter seals between the electrode and membrane. The results obtained were identical to those of muscles without collagenase treatment.
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Increased Numbers of Thoracic Dorsal Root Axons in Rats Given Antibodies to Nerve Growth Factor

Abstract. *Sensory axons were counted in untreated 1-month-old rats and in littermates that were injected with antibodies to nerve growth factor. There were 45 percent more unmyelinated and 17 percent more myelinated axons in dorsal roots of the fifth thoracic spinal segment in treated rats. This suggests that the number of sensory axons can be changed by postnatal inactivation of nerve growth factor.*

Nerve growth factor (NGF) affects the growth and development of sensory and sympathetic neurons (*1, 2*). On the sensory side, this compound is necessary for the survival of dorsal root ganglion cells and the outgrowth of sensory processes in vitro (*3*). Furthermore, it has been suggested that NGF is transported from peripheral endings to appropriate neuronal cell bodies and that this serves as a signal that innervation is being maintained (*4*). In vivo, treatment with antibodies to NGF before birth reduces the number of primary sensory cells (*5*), but there is no demonstrable postnatal effect on these cells (*5, 6*). It was recently discovered, however, that postnatal manipulation of NGF levels changes the number of axons in a peripheral nerve (*7*) and in the dorsal root after spinal injury (*8, 9*). This suggests that sensory axons might be influenced by postnatal inactivation of NGF even if the number of sensory cells does not change.

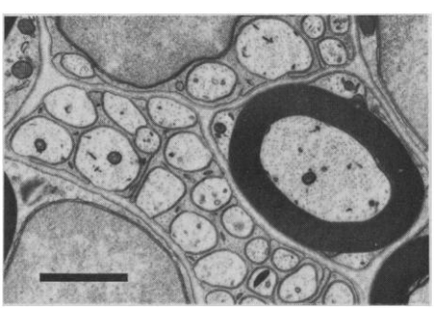

Fig. 1. Electron micrograph of a cross section of a dorsal root, showing a myelinated axon (right) surrounded by smaller, unmyelinated axons. Because of their distinctive morphology, myelinated and unmyelinated axons can readily be counted with the electron microscope. Scale bar, 1 μm.

Pregnant Sprague-Dawley rats were obtained from Texas Inbred Mouse Company. When the pups were born they were given daily injections (3 μl/g subcutaneously) of antibodies to NGF (anti-NGF; undiluted rabbit antiserum) at a site near the dorsal fat pad for 4 weeks. The anti-NGF was prepared against the purified mouse β-NGF subunit as described by Beck and Perez-Polo (*10*). In bioassays of embryonic chick sensory ganglia or of human neuroblastoma cells, a 1:500 dilution of the

antiserum was found sufficient to "block" exogenously applied NGF at concentrations containing one biological unit of activity per milliliter. Consequently, the use of undiluted antisera in these experiments was deemed sufficient to block endogenous levels of NGF, which are known to be in the nanogram range (*11*). As a control, samples of whole serum from the same rabbits that were later used to produce the anti-NGF were given to rats as described above. Axon numbers in these rats were the same as in normals (*8*).

After 28 days the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg) and perfused with 0.9 perent NaCl containing 0.2 ml of 1.0 percent $NaNO_2$ and 200 U of heparin per 100 cm^3. When the effluent from the right auricle was free of blood, the perfusion fluid was changed to 3 percent glutaraldehyde, 3 percent paraformaldehyde, and 0.1 percent picric acid in 0.1*M* cacodylate buffer (*p*H 7.4). After 1 to 3 hours, the dorsal roots were removed and placed in the aldehyde solution overnight. The following day the tissue was placed in a solution of 1 percent osmic acid and 1.5 percent potassium ferricyanide (*12*) in 0.1*M* cacodylate buffer (*p*H 7.4) for 1 to 2 hours. The tissue was then stained in 0.5 percent uranyl acetate and embedded in a mixture of Epon and araldite. Thin sections were cut with a diamond knife, placed on single-hole grids covered with Formvar film, stained with 0.1 percent lead citrate or left unstained, and examined in a Philips 300 or 301 electron microscope (Fig. 1). All myelinated and unmyelinated axons were counted. Error in repeated counts of the tissue was ±3 percent. Statistical significance ($P < 0.05$) was determined by Student's *t*-test.

Counts of axons in dorsal roots of the fifth thoracic spinal segment (T5) of untreated rats and anti-NGF–treated littermates are presented in Table 1. There were, on average, 1358 myelinated and 4097 unmyelinated axons in the roots from untreated rats and 1595 myelinated and 5944 unmyelinated axons in the same roots from treated rats. Thus, there were 17 percent more myelinated and 45 percent more unmyelinated axons in the roots from treated animals. These differences are significant at $P < 0.017$ and $P < 0.0001$, respectively.

To our knowledge, this is the first demonstration of a postnatal change in the number of dorsal root axons caused by manipulation of NGF only. The observation that axonal numbers increase after anti-NGF treatment is surprising, because it was previously reported that the number of cells or processes increases when NGF is present and decreases when NGF is not present or is inactivated (*2, 5, 6*). As an explanation, we suggest that NGF acts as a signal from a target cell to the neuron indicating that normal connections are being maintained (*3*). Thus, withdrawal of NGF might indicate that axonal connections are not present, which leads to compensatory sprouting (*4, 9, 13*). Since the axons in the dorsal root are the structural basis of dermatomes, studies are needed to determine whether the qualities of sensation and the size of dermatomes change as a result of such treatment. We also need to determine whether these changes occur in other spinal segments and in other mammals and whether they still occur if the anti-NGF is administered earlier or later in development. Our findings raise the possibility of increasing the number of sensory axons by a noninvasive postnatal technique.

Table 1. Counts of myelinated and unmyelinated axons from rats treated with anti-NGF and from untreated littermates. The counts were made from the left and right dorsal roots of the fifth thoracic segment.

Untreated rats		Anti-NGF–treated rats	
Myelinated axons	Unmyelinated axons	Myelinated axons	Unmyelinated axons
Left dorsal root			
1236	4276	1660	6053
1263	3364	1319	5343
1520	4135	1496	6868
1271	4066	1928	6073
Right dorsal root			
1371	3574	1678	5622
1439	4643	1794	6498
1381	4295	1624	5979
1379	4423	1260	5116
Mean ± standard error			
1358 ±34	4097 ±152	1595 ±80	5944 ±204

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Mating in Bighorn Sheep: Multiple Creative Male Strategies

Abstract. *Rocky Mountain bighorn rams obtained copulations by defending single estrous ewes (tending), fighting tending rams for temporary access to defended ewes (coursing), or moving and holding ewes away from other rams beyond the periphery of a traditional tending area (blocking). Coursing and blocking illustrate a feature of many male alternative mating strategies: the ability of males regularly to create mating opportunities.*

A primary and one or more alternative strategies are typically distinguished when different male mating strategies coexist within populations (*1*). Males that use primary strategies generally "create" mating opportunities with some form of sustained intrasexual aggression, of which territorial and harem defense are examples. In contrast, males that use diverse alternative strategies are often depicted as "opportunists," taking advantage of mating chances provided by various extrinsic causes, such as a territorial dispute that leaves females unguarded (*1*). I now present evidence that Rocky Mountain bighorn rams (*Ovis canadensis canadensis*) mate in three distinct ways. A primary strategy, tending, coexists with two alternatives, blocking and coursing. Most males both