Neurotrophic Influence on Lobster Skeletal Muscle

Abstract. *The correlation between histochemical properties of muscle fibers and the pattern of innervation by the two motor neurons was studied in the asymmetric claw closer muscles of the lobster. The closer muscle of the cutter claw is composed of 65 percent fast muscle fibers and 35 percent slow muscle fibers, whereas that in the crusher claw has all slow muscle fibers. In both claws, myofibrillar adenosinetriphosphatase activity was independent of the pattern of innervation. Oxidative capacity, as measured by reduced nicotinamide adenine dinucleotide activity, was correlated with motor axon presence: Muscle fibers innervated solely by the "fast" motor axon had low oxidative capacity, muscle fibers receiving only the slow motor axon had very high oxidative capacity, and fibers innervated by both axons had intermediate properties. The data suggest that the motor neurons may exert trophic influences that control certain muscle fiber properties but not others.*

A complex dynamic relationship has been established to exist between a neuron and the structure it innervates, be it another neuron, a muscle, or a gland. In addition to the well-studied processes concerned with generation of postsynaptic potentials (PSP's), other more subtle "trophic influences" appear to control certain postsynaptic properties. Indeed, the integrity of the innervated structure depends on the presence of these trophic influences (*1*). A clear demonstration of this dependence can be obtained when the nerve to a skeletal muscle is cut or if function of the nerve is otherwise disturbed (*2*); in either event, the muscle will atrophy. Furthermore, the motor nerve will influence specific properties of vertebrate skeletal muscle; if the nerve to a slow muscle is transplanted to a fast muscle, some properties of the muscle will transform to those resembling a slow muscle (*3*). Although the demonstration of these trophic influences is clear, their exact nature is as yet uncertain (*4-6*).

We have been investigating nerve-muscle interactions during development and growth of the lobster. This system provides an opportunity to study fast and slow fibers from muscles whose properties are not fixed at the time of hatching (*7*). The muscle is innervated by only two identifiable motor neurons, so it is possible to determine which characteristics of the muscle are correlated with the pattern of innervation and which are apparently independent of this influence. We report that the oxidative capacity of lobster skeletal muscle is correlated with the pattern of innervation by the fast and slow motor neurons (*8, 9*). In contrast, specific activity of the myofibrillar adenosinetriphosphatase (*10*) has no obvious correlation with the pattern of innervation. The results are consistent with a hypothesis that the motor neurons may exert a trophic influence, perhaps chemical in nature or by activity, on oxidative metabolism but has little or no influence on sarcomere length or specific activity of myofibrillar adenosinetriphosphatase.

Experiments were performed on lobsters (*Homarus americanus*) ranging in size from 50 to 400 g (*11*). Most of the electrophysiological work was performed on larger animals, and histological results were obtained from smaller animals. Innervation patterns of different-sized animals did not differ. The primary objective was to correlate properties of muscle fibers, including specific activity of myofibrillar adenosinetriphosphatase and oxidative capacity, with the pattern of innervation by the motor axons. To determine the pattern of innervation, animals were induced to autotomize a claw. The two motor axons (*12*) were isolated in the carpopodite; motor axon action potentials were monitored with a suction electrode, and intracellular recordings were made from individual muscle fibers of the closer muscle according to conventional techniques (*13*). Careful placement of the stimulating electrodes permitted selective excitation of either motor axon alone. By removing pieces of exoskeleton from the propus and monitoring electrical activity in the underlying fibers, we were able to determine the distribution of the motor axons to all areas (deep and superficial) of the closer muscles (*14*).

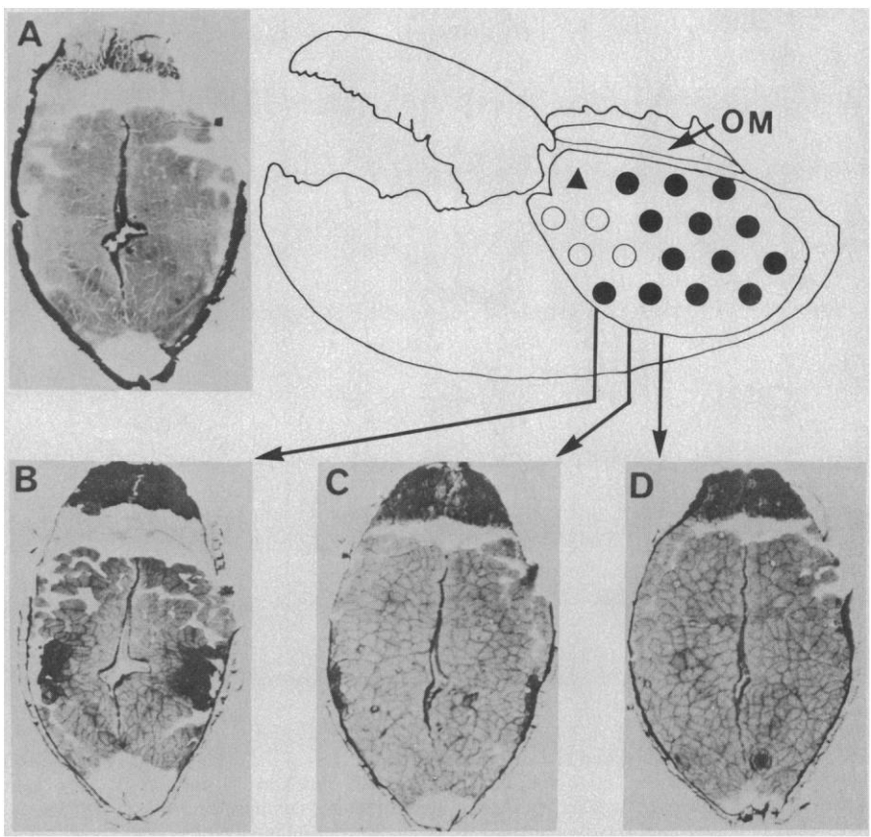

Fig. 1 Pattern of innervation and histochemical characteristics of the closer muscle of the crusher claw. (Top right) Diagram of crusher claw showing the opener (*OM*) and closer muscle. The closer muscle is composed entirely of muscle fibers with long sarcomeres innervated by the "fast" motor axon (▲), the "slow" motor axon (○), or by both motor axons (●). Serial frozen sections were cut from the entire muscle, and alternate sections were stained for myofibrillar adenosinetriphosphatase (A) or NADH diaphorase (B to D) activity (A and B are from same plane of section). The closer muscle stained uniformly light for adenosinetriphosphatase activity, regardless of the area from which the sections were taken. The NADH diaphorase activity was high for all fibers but was highest in the distal region (B), where the fibers are innervated solely by the slow motor axon. Thus, the oxidative capacity is related to the pattern of innervation by the motor neurons, whereas the adenosinetriphosphatase activity is not.

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Muscle histochemistry was investigated in intact claws frozen in isopentane cooled by liquid nitrogen, and then sectioned in a cryostat. Serial sections were taken from cutter and crusher claws and stained for adenosinetriphosphatase (*15*) or reduced nicotinamide adenine dinucleotide (NADH) diaphorase, the former to determine the relative activity of the myofibrillar adenosinetriphosphatase (*16*) and the latter to measure the oxidative capacity of the muscle fibers (*8*).

The pattern of innervation by the two motor axons followed a distinct configuration for each of the two claw closer muscles (Figs. 1 and 2). The majority of muscle fibers in the cutter claw receive the fast motor axon, and the slow motor axon serves primarily the ventral bundles of slow muscle fibers. In the crusher claw, both the fast and slow motor axons serve more than 67 percent of the muscle fibers. The synaptic properties of the axons are significantly different; in general, the fast axon in both claws had excitatory PSP's ranging from 1 to 5 mV at 1 Hz stimulation, which facilitated twofold at 10 Hz. The PSP's from the slow axon were smaller at 1 Hz (< 0.1 to 2 mV), but they facilitated about sixfold at 10 Hz (*17*). For both axons, the largest PSP's were found in muscle fibers innervated exclusively by that axon.

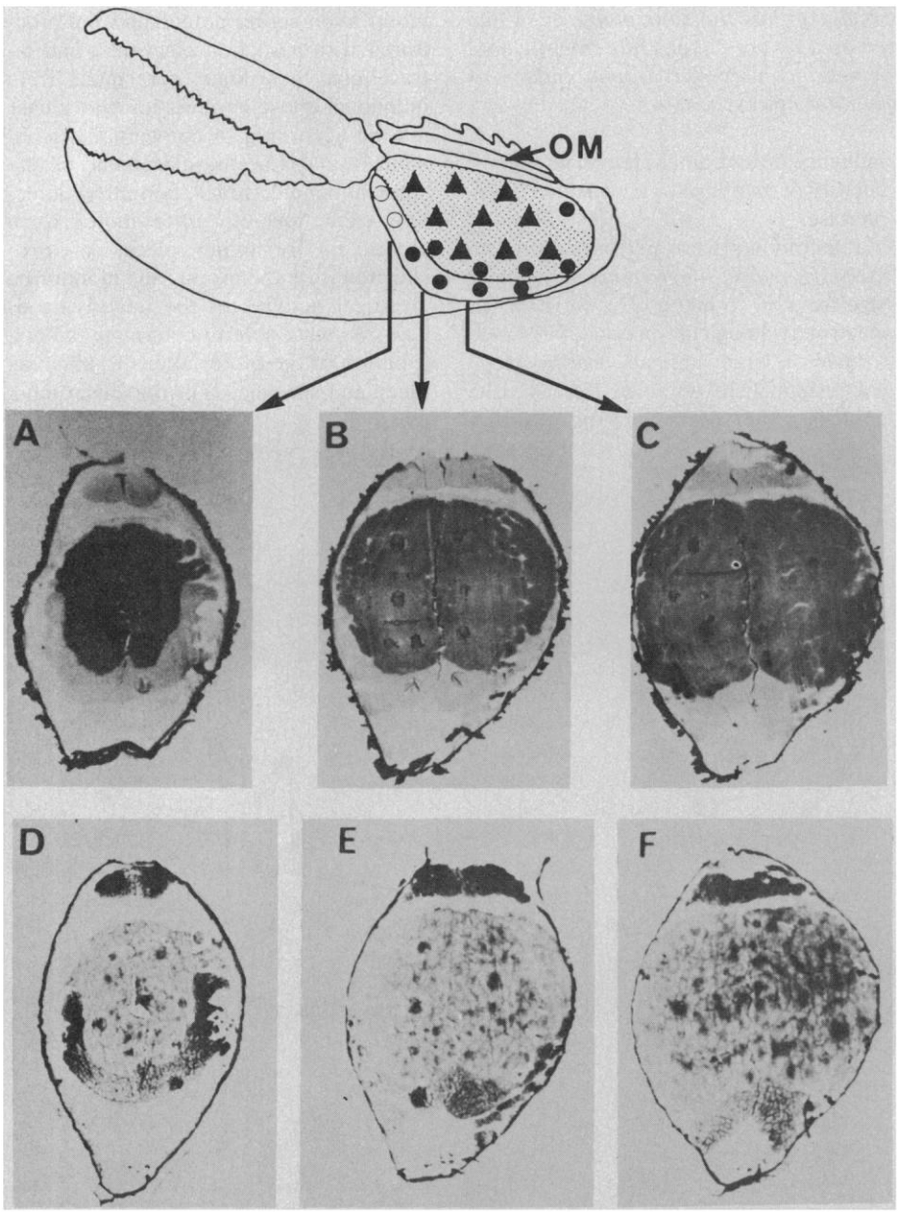

Fig. 2 Pattern of innervation and histochemical characteristics of the closer muscle of the cutter claw. (Top) Diagram of the cutter claw depicting the large closer muscle and the smaller opener muscle (*OM*). The closer muscle has a large dorsal bundle, composed chiefly of fast muscle fibers with short sarcomeres (stippled area) and a smaller ventral bundle of slow fibers with long sarcomeres. Of the two motor axons to the closer muscle, the "fast" motor axon innervates the dorsal bundle (▲), the slow axon innervates some distal slow fibers (○), and the ventral slow fibers and some dorsal fibers receive both axons (●). Serial frozen sections were cut from the entire muscle, and alternate sections were stained for myofibrillar adenosinetriphosphatase (A to C) or NADH diaphorase activity (D to F). Pairs of sections are shown from the medial (C to F), middistal (B to E), and distal areas (A to D) (arrows). On the sections stained for adenosinetriphosphatase, the fast fibers stained darkly and the more ventral slow fibers stained lightly. In the sections stained for NADH diaphorase, the fast fibers stained lightly and the slow fibers more darkly, particularly in the sarcolemmal region. In the distal region (D), where the slow muscle fibers are innervated solely by the slow motor neuron, they stained more darkly, revealing a higher oxidative capacity than the more medial and proximal slow fibers.

The histochemical staining properties of the closer muscles exhibited considerable heterogeneity when stained for NADH diaphorase, an indicator of oxidative capacity (*18*). The closer muscle of the crusher claw is composed entirely of long-sarcomere (6 to 12 μm) slow fibers (*19*). In general the staining intensity of these fibers was related to the presence or absence of innervation by the fast motor axon. Where the fast motor axon innervated the muscle fibers (along with the slow axon), the staining intensity was low. However, in the distal portion of the closer muscle, where fibers are innervated solely by the slow motor axon (Fig. 1), there was a very intense staining for NADH diaphorase activity, suggesting a very high oxidative capacity. A similar darkly staining area was present in the proximal region of the muscle, where a bundle of fibers is also innervated solely by the slow axon (not shown).

In the cutter claw, the NADH diaphorase staining pattern was again correlated to the pattern of innervation. The dorsal fibers are all fast with short sarcomeres (2 to 4 μm) and innervated solely by the fast axon; these fibers stained lightly for NADH diaphorase activity. The majority of the ventral slow fibers receive both axons; these stained with an intermediate intensity. In the distal region, a bundle of slow fibers receiving only a slow motor axon (Fig. 2) stained darkly for NADH diaphorase (Fig. 2D).

The staining pattern for myofibrillar adenosinetriphosphatase was quite different and is apparently independent of the pattern of innervation. Only two staining patterns were observed for this enzyme in animals with mature claws; fast muscle (short sarcomere) stained darkly, whereas slow muscle (long sarcomere) stained lightly. This was true even for slow muscle fibers in the crusher claw, which are innervated solely by

the fast axon (Fig. 1A). These staining properties could be due either to differences in the specific activity of the myofibrillar adenosinetriphosphatases in fast and slow fibers or to differences in the amount of enzyme present. These possibilities were tested by dissecting out bundles of fast and slow muscle fibers from representative areas of both claws. In agreement with previous findings on lobster abdominal muscle (*16*), specific activity of the myofibrillar adenosinetriphosphatase in fast muscle was two to four times that in slow muscle (*20*).

These results suggest that the muscle fiber properties of myofibrillar adenosinetriphosphatase activity, sarcomere length, and probably related characteristics (*21*) are not correlated in any simple way with the pattern of innervation by the fast and slow motor neurons. We have observed slow muscle (long sarcomere and low adenosinetriphosphatase activity) innervated by the slow axon alone, by the fast axon alone, or by both axons (Fig. 1). Fast muscle can be innervated by the fast axon alone or by both axons; we have yet to observe fast muscle fibers receiving only the slow axon. Furthermore, these muscles have been demonstrated to be not under rigid genetic control, and their properties can be transformed during early juvenile stages (*7*). The factors controlling these related properties have yet to be determined, but as in vertebrate muscle (*5*), activity may play an important role (*22*).

The oxidative capacity of the muscle fibers is not directly correlated with the muscle fiber type (based on adenosinetriphosphatase activity and sarcomere length). In accord with previous findings (*9*), we found that this property is highly variable even among fibers with similar sarcomere lengths. Thus, oxidative capacity appears to be proportional to the degree of innervation by the slow motor neurons. The amount of excitatory PSP depolarization by the slow axon is correlated with this metabolic profile. The largest PSP's evoked by slow axons are found in the distal fibers, which receive only this motor axon; they probably receive more depolarization from this axon than those fibers which also receive the fast motor axon.

These findings lead to another important conclusion regarding crustacean skeletal muscle, namely, that there may be greater heterogeneity of fiber types within a muscle than had been previously appreciated. Crustacean skeletal muscles are known for great species-to-species diversity, but they have generally been characterized as fast, slow, or intermediate for a given species (*23*). Only rarely has heterogeneity of some properties been described among what appeared to be an otherwise uniform population of muscle fibers (*19, 24*). We have demonstrated that some of this diversity may depend on the pattern of innervation by the motor neurons, but that other features are apparently independent of this influence (*25*).

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References and Notes

1. For reviews, see L. Guth, *Physiol. Rev.* **38**, 177 (1968); A. J. Harris, *Annu. Rev. Physiol.* **36**, 251 (1974); E. Gutman, *ibid.* **38**, 177 (1976).
2. This can be accomplished in a variety of ways, including tenotomy, immobilization, and spinal cord isolation (*1*).
3. These "cross reinnervation" experiments also work for transplanting a "fast" nerve to a slow muscle, but the transformation is usually less complete (*1*).
4. There is evidence that muscle activity is important (*5*), but release of specific chemicals from the presynaptic neuron has also been implicated (*6*).
5. G. Vrbova, *J. Physiol. (London)* **169**, 513 (1963); D. Salmons and G. Vrbova, *ibid.* **201**, 535 (1969); L. Guth, F. J. Samaha, W. Albers, *Exp. Neurol.* **26**, 216 (1970).
6. F. Kauffman, E. X. Albuquerque, J. E. Warnick, S. R. Max, *Exp. Neurol.* **50**, 60 (1976).
7. F. Lang, C. K. Govind, W. J. Costello, *Science* **201**, 1037 (1978).
8. Generally, slow muscles have high oxidative, low glycolytic capacity, whereas fast muscles have low oxidative, high glycolytic capacity. This is true for vertebrate (*1*) and crustacean muscle, though the differences among crustacea are less pronounced (*9*). In mammals, the metabolic profile can be altered by changing the activity of a muscle [D. Pette, M. E. Smith, H. W. Staudte, G. Vrbova, *Pfluegers Arch.* **338**, 257 (1973)].
9. I. Hajek, N. Chari, A. Boss, E. Gutmann, *Physiol. Bohemoslov.* **22**, 603 (1973).
10. The activity is measured as micrograms of inorganic phosphate liberated per gram of protein per minute. Fast muscle has a higher specific activity than slow muscle for vertebrates [M. Barany, *J. Gen. Physiol.* **50**, 197 (1967)] and crustaceans (*9*).
11. Animals of 350 to 400 g were obtained from local waters and kept in 100-gallon tanks provided with fresh, running seawater at ambient temperatures. Smaller animals of 50 to 100 g were raised from postlarval stages as previously described [F. Lang, *Aquaculture* **6**, 389 (1975)]. Animals in this size range have fully differentiated claws [C. K. Govind and F. Lang, *Biol. Bull. (Woods Hole, Mass.)* **154**, 55 (1978)].
12. There are two motor axons to each closer muscle, one fast and one slow [C. A. G. Wiersma, *Arch. Neerl. Zool.* **11**, 1 (1955)]. At 15°C, the former has a conduction velocity (CV) of 11 m/sec, while the slow axon has a CV of 9 m/sec at 15°C. Each muscle also received an efferent inhibitor axon which innervated very few muscle fibers, primarily those which are served only by the slow excitor [R. Hill and F. Lang, *J. Exp. Zool.* **208**, 129 (1979)].
13. C. K. Govind and F. Lang, *Experientia* **32**, 1170 (1976).
14. W. J. Costello and F. Lang, *Biol. Bull.* **156**, 179 (1979); W. J. Costello, R. Hill, F. Lang, in preparation. The closer muscles are divided into distinct bundles of homogeneous fiber types. The bulk of the muscles is grouped into a large dorsal and smaller ventral bundle attached, in pinnate fashion, to the apodeme. The dorsal bundle is composed of fast muscle fibers in the cutter claw and slow muscle fibers in the crusher. The ventral bundle is slow in both claws. In the proximal region are three small bundles, two dorsal and one ventral (not shown in the figures), which parallel the larger bundles in terms of fiber properties; that is, the dorsal bundles are fast in the cutter, whereas all others are composed of slow muscle fibers.
15. Methods for adenosinetriphosphatase were similar to those described by H. A. Padykula and E. Herman [*J. Histochem. Cytochem.* **3**, 161 (1955); *ibid.* **3**, 170 (1955)] for NADH diaphorase [M. M. Nachlas, D. G. Waler, A. M. Seligman, *J. Biophys. Biochem. Cytol.* **4**, 29 (1958)]. Detailed methods are described elsewhere [M. Ogonowski and F. Lang, *J. Exp. Zool.* **207**, 143 (1979)]. To determine that the adenosinetriphosphatase activity was primarily myofibrillar, the following controls were run: (i) Incubation without adenosine triphosphate resulted in no staining; (ii) substitution of glycerolphosphate for adenosine triphosphate (test for nonspecific phosphatase) resulted in no staining; (iii) adding sodium azide (a blocker of mitochondrial adenosinetriphosphatase) did not affect distinguished differences in staining patterns among muscles [T. Ogata and M. Mori, *J. Histochem. Cytochem.* **12**, 183 (1964); A. J. Vitale and D. R. Stokes, *Am. Zool.* **17**, 899 (1977); D. R. Stokes, A. J. Bitale, C. R. Morgan, *Cell Tissue Res.* **198**, 175 (1979)]. However, the heterogeneity reported in some muscles remains unexplained.
16. W. Lehman and A. G. Szent-Györgi, *J. Gen. Physiol.* **66**, 1 (1975).
17. C. K. Govind and F. Lang, *J. Exp. Zool.* **190**, 281 (1974).
18. Preliminary results with histochemical staining for succinic dehydrogenase, another indicator of oxidative capacity, gave results identical to those described for NADH.
19. S. S. Jahromi and H. L. Atwood, *J. Exp. Zool.* **176**, 475 (1971); L. Goudey and F. Lang, *ibid.* **189**, 421 (1974).
20. The specific activity was measured as moles of inorganic phosphate per minute per milligram of protein. The values for fast muscle were 0.71 ± 0.16; for slow muscle they were 0.49 ± 0.12 (cutter) and 0.23 ± 0.08 (crusher) (*16*).
21. H. L. Atwood, in *The Structure and Function of Muscle*, G. Bourne, Ed. (Academic Press, New York, 1972), p. 421.
22. Intracellular recording from closer muscle fibers of intact, semirestrained lobsters suggests that the fast axon of the crusher claw may fire tonically but the fast axon in the cutter does not [R. Hill, W. J. Costello, F. Lang, *Am. Zool.* **17**, 904 (1977)].
23. Fast muscle generally has short sarcomeres, a low ratio of actin to myosin, a well-developed tubular system and sarcoplasmic reticulum, excitable membranes, few mitochondria, and little glycogen. Slow muscle usually has the opposite characteristics, and intermediate fibers share some properties of each category.
24. G. Hoyle, *J. Exp. Zool.* **185**, 97 (1973); ——— and P. A. McNeill, *ibid.* **167**, 487 (1968).
25. Preliminary results of these studies have been reported [F. Lang, W. J. Costello, C. K. Govind, *Proc. Congr. Int. Union Physiol. Sci.* **13**, 426 (1977); F. Lang, W. J. Costello, M. Ogonowski, B. Roehrig, *Biol. Bull. (Woods Hole, Mass.)* **153**, 434 (1977)].
26. We thank A. G. Szent-Györgyi for assistance with adenosinetriphosphatase determinations and for invaluable discussions. H. L. Atwood, C. K. Govind, M. P. Charlton, and H. Silverman provided helpful criticisms of the manuscript. Special thanks go to L. Hill for expert help. Supported by grants from the National Science Foundation, the National Institutes of Health, and Boston University Graduate School. F. L. was in receipt of a Research Career Development Award (NS-00307) and J.S. is an NIH predoctoral trainee (GM 7122). While this report was in preparation Dr. Fred Lang was killed in an automobile accident. His death brought short a highly productive career in crustacean neuromuscular physiology.
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