as for light-scattering experiments and the assay was performed in a temperature-controlled room at 37.5°C. The videotape was later analyzed to find the swimming speeds of 563 separate spermatozoa, from which a separate swimming speed distribution was constructed.

In performing the splines analysis, the optimum number of parameters $[P(V_N)]$ was selected by minimizing the error $\chi^2 = [g^{(2)}(\tau)_{exp} - g^{(2)}(\tau)_{theor}]^2$. The results of the splines analysis of the data of Fig. 1 are shown in Fig. 2 along with the results of the videomicroscopic assay. Figure 2 shows that for speeds in the range 0 to 80 μm/sec, the splines and videomicroscopic assays agree rather well. However, the "high-speed tail" extending out to speeds well beyond 150 μm/sec is apparently not associated with the translational motion. A plausible explanation for this is that the rotational effects cannot be completely neglected, and their contributions are evident in the high-speed portions of the distribution curve. Craig *et al.* (*9*) state that at a scattering angle of 15°, the electric field correlation function for the large disk-shaped heads of bull spermatozoa (1 by 5 by 9 μm) is dependent entirely on the rotational motion of the head. The head of the human sperm is less anisotropic (2 by 2 by 4 μm) and its correlation data should reflect a mixture of rotational and translational motion. In Table 1 we give the numerical results of our analysis, together with the results of other investigators.

In conclusion, we suggest that photon correlation spectroscopy can provide a fast, reasonably accurate means of establishing the swimming speed distribution of motile sperm through on-line computer analysis utilizing the method of splines. Samples prepared by dilution in seminal plasma circumvent problems associated with multiple scattering while avoiding the reduction of viscosity due to dilution with aqueous media that leads to increased wobbling and distorted swimming speed distributions.

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Remodeling of Multiterminal Innervation by Nerve Terminal Sprouting in an Identifiable Lobster Motoneuron

Abstract. *A single motoneuron provides multiterminal innervation to the limb accessory flexor muscle in lobster. Its nerve terminals and synapses relocate to more distal sites during primary development and growth beyond sexual maturity. This remodeling of multiterminal innervation occurs by sprouting of nerve terminals and synapses from preexisting ones.*

The development of innervation occurs by the sprouting of intact axons at their peripheral fields (*1*). Although such sprouting has been found largely during primary development, there is evidence that it occurs in mature stages as well, where it may replace old nerve terminals (*2, 3*). This raises the intriguing possibility that nerve terminals in the periphery and the central nervous system (CNS) are in a dynamic state because of constant growth and restructuring by the sprouting mechanism. We have found a remodeling of the multiterminal innervation arising from a single identifiable lobster motoneuron during primary development. It consists of a shift in nerve terminals and synapses to the ever more distal and finer branches of the axon by sprouting from preexisting terminals and synapses. Such sprouting also occurs in several sizes of large and chronologically older adult lobsters, which suggests that the restructuring of multiterminal innervation occurs throughout life.

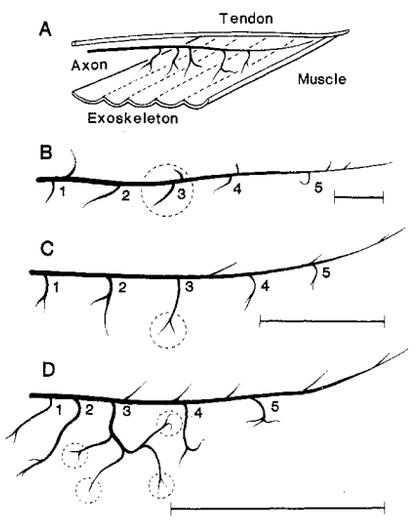

Fig. 1. Location of five primary branches on the exoskeletal side of the single excitor axon to the DAFM in an adult lobster (A) and their development as seen in a 1-day-old (first stage) larva (B), a 1-year-old (twelfth stage) juvenile (C), and a 5- to 7-year-old adult (D). Primary branches are numbered 1 to 5, proximal to distal. Encircled areas show the typical location of nerve terminals and neuromuscular synapses in the comparable branch 3 region of each developmental stage. Scale bars (B) 0.01 mm; (C) 1 mm; and (D) 10 mm.

We studied multiterminal innervation to the accessory flexor muscle in the walking legs of lobster (*Homarus americanus*) because it is supplied by a single excitatory and inhibitory axon (*4*). The branching pattern of the excitor axon can be easily distinguished from that of the inhibitor in preparations stained with methylene blue, as the excitor has a larger diameter (*5*). Furthermore, the nerve terminals of each axon have synaptic vesicles that differ in shape with aldehyde fixation: vesicles of excitatory terminals are spherical, those of inhibitory terminals, ellipsoidal (*6*). These features enabled us to identify the single excitatory neuron to the distal accessory flexor muscle (DAFM) in the first walking leg and to reconstruct its branching pattern by methylene blue staining in juvenile and adult lobsters and by serial section electron microscopy in a larval lobster (*7*). The latter technique was also used to identify and locate nerve terminals and synapses in all of the lobsters examined.

At a gross level, the development of

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multiterminal innervation is dramatically seen when the branching pattern of the single excitatory axon is compared among larval, juvenile, and adult lobsters (Fig. 1). In each stage, the axon traverses the width of the muscle close to the tendon and branches on both sides (Fig. 1A). The primary branches on the exoskeletal side are comparatively larger and consistently five in number at each stage (Fig. 1, B to D); those on the tendinal side are smaller and variable in number. The five primary branches on the exoskeletal side provide most of the innervation, and it is their development that is of particular interest. In the first larval stage these five branches occur as short sprouts of the parent axon (Fig. 1B) with no evidence for further branching. The comparable five primary branches have acquired secondary branches in a 1-year-old juvenile (Fig. 1C) and tertiary branches in an adult (Fig. 1D). Consequently, the branching pattern of the motoneuron develops in complexity from larval to adult stages while retaining the five primary branches.

During development of this branching pattern there is a concomitant shift in the location of neuromuscular terminals and synapses at each stage. This shift is illustrated by encircling the location of terminals and synapses in one area of the axonal branching at each developmental stage in Fig. 1. In the larva, nerve terminals and synapses are found in approximately equal numbers on the axon and the primary branches. In the juvenile, they are not found on the parent axon but are restricted to the primary and secondary branches; in the adult, however, they occur on secondary, tertiary, and finer branches but not on the parent axon or primary branches. The shift in neuromuscular terminals and synapses to the ever more distal points of the axonal branching system denotes a major remodeling of multiterminal innervation during primary development.

The manner in which this remodeling occurs is suggested from serial section electron microscopy of the innervation at each developmental stage. In multiterminal innervation, axonal regions are distinguished from nerve terminal regions. The former are usually surrounded by Schwann cells and connective tissue, have numerous neurotubules, but lack synapses and synaptic vesicles, and the latter are in contact with granular sarcoplasm of the muscle, lack neurotubules, but have synapses with aggregations of synaptic vesicles (*8*). In the first larval stage, according to these criteria, half the length of the parent axon over the muscle was differentiated into nerve terminals; it was only from these areas that the axon sprouted primary branches (Fig. 2, A and B). All primary branches along the entire length of the axon on the muscle arose in this manner. Since the larval axon is actively developing its branching at this stage (*9*), these findings strongly suggest that sprouting in a lobster axon occurs from its nerve terminals.

Additional support for this hypothesis comes from finding that the primary sprouts are themselves terminals with distinct synapses and aggregations of synaptic vesicles (Fig. 2, C and D). In this condition, the primary sprouts provide half of the innervation to the larval DAFM. However, as the muscle increases in size, a concomitant increase in innervation occurs via the five primary branches on the exoskeletal side in juvenile and adult DAFM's (Fig. 1, C and D). Since the forerunners of these primary branches are presumably the five primary sprouts on the exoskeletal side of the larval axon (Fig. 1B), these larval sprouts represent growing points.

Similar growing points were also found in the juvenile innervation that was serially sectioned for 36 μm, except that here they were restricted to regions of the primary axonal branches that had differentiated into synaptic terminals. In the adult, serial sectioning of the innervation originating from three of the five primary branches (branches 1, 2, and 5 of Fig. 1C) showed the growing points in secondary, tertiary, and finer branches, again in synaptic terminal areas. The sprouting of new synapses and terminals from preexisting ones and the subsequent transformation of the original synaptic terminal areas into axonal areas represents a major restructuring of multiterminal innervation during primary development.

Does nerve terminal sprouting and the consequent remodeling of innervation occur beyond sexual maturity in lobsters? Since lobsters lack a terminal molt and continue growing throughout life, we examined three large adult lobsters weighing 4.9, 7.8, and 9.2 kg. Lobsters are sexually mature at 5 to 7 years of age when they weigh approximately 500 g (*10*). As the animals do not double their weight at each molt and as the frequency of molting declines with age, the large adults we selected were consid-

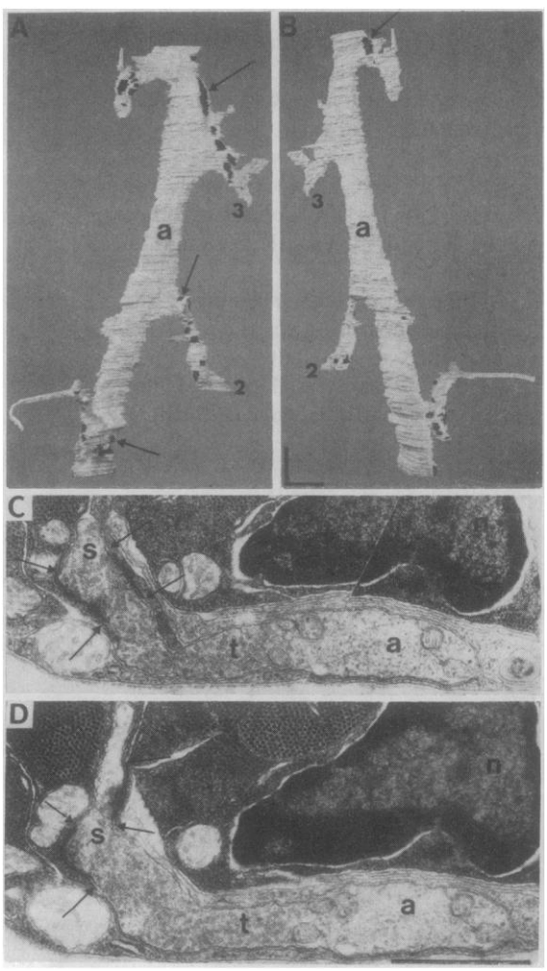

Fig. 2. (A and B) Opposite views of a three-dimensional reconstruction of part of the DAFM excitor axon (*a*) in a 1-day-old larval lobster, showing locations of synapses (black areas) and of primary branches 2 and 3 (compare Fig. 1B). Primary branches originate from synapse-bearing areas (arrows) of the axon and are themselves nerve terminals filled with synapses. Horizontal and vertical scale, 3 μm. (C and D) Micrographs from serial reconstruction A and B showing nerve terminal (*t*) at one end of ovoid-shaped axon (*a*) sprouting a primary branch (*s*). The branch is filled with synaptic vesicles, has synapses (between arrows) and elongates as a nerve terminal. The nucleus (*n*) is shown at the upper right. Magnification, ≈ ×18,500; scale bar, 1 μm.

erably older, ranging approximately from 20 to 80 years (*11*). In these older animals as well, serial sectioning of the innervation provided by two of the five primary branches revealed outgrowths from synaptic terminals. Indeed, in the 4.9-kg lobster, sprouting occurred from a synapse itself (Fig. 3), a striking demonstration of the thesis that sprouting in this lobster motoneuron is from synaptic terminals. It was more usual to find finger-like extensions arising from areas adjacent to synapses in a nerve terminal. In any case, for a total of 90 μm of innervation that was serially sectioned in these large adults, we found such outgrowths only from synaptic terminal areas and not from axonal areas. This does not preclude the possibility that growth may occur from growth cones, described in other animals as the tip of an axon or its branch (*12*). Our data, however, show that the single excitor axon to the limb accessory flexor muscle in lobster sprouts new innervation from its synaptic terminals both during primary development and during subsequent growth in adults.

We have found changes in synaptic transmission from terminals of this same motoneuron on the proximal half of the bipartite accessory flexor muscle (*13*). Here the mean number of quanta doubled between comparable synapses of small (300 g) and large (3 kg) lobsters, compensating for the twofold increase in fiber diameter between the two size classes. Our finding of nerve terminal sprouting in large and presumably old lobsters provides a structural correlate for the increase in synaptic transmission with growth.

It is interesting that sprouting in the lobster motoneuron is restricted to the synapse or areas immediately adjacent to it, since the synapse is already a highly specialized area for chemical transmission. Transmitter release at the lobster neuromuscular junction may be by exocytosis of vesicles (*14*) as at the frog neuromuscular junction (*15*), where the vesicles fuse with the synaptic membrane and re-form from immediately adjacent areas. The perpetual addition and withdrawal of membrane in the synaptic region render it a prime target for sprouting.

Finally, the remodeling of the innervation by sprouting (this report) and by the increase in transmitter output (*13*) during growth beyond sexual maturity shows the ongoing nature of neuronal plasticity in a single identifiable lobster motoneuron. In contrast, the majority of previous demonstrations of plasticity have been on populations of neurons in the CNS (*2*, *16*). Since the motoneuron to the lobster accessory flexor muscle mimics central neurons in having multiple converging inputs, it can serve as a simple model system for studying neuronal plasticity.

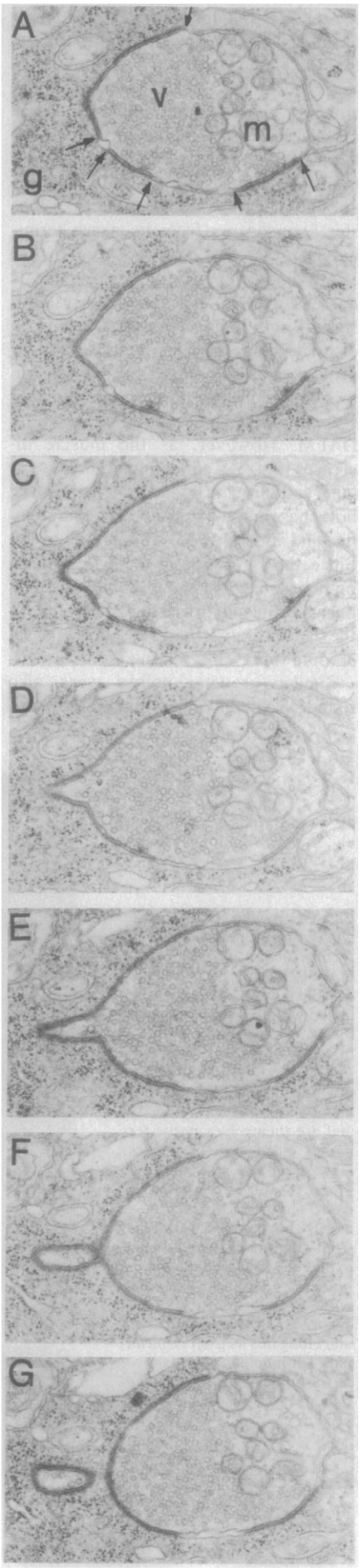

Fig. 3. Serial micrographs of an excitatory nerve terminal in DAFM of a 4.9-kg lobster, showing the largest of three synapses (between arrows) sprouting a small new terminal. Abbreviations: *v*, synaptic vesicles; *m*, mitochondria; *g*, muscle granular sarcoplasm. Magnification, ×32,500; scale bar, 1 μm.

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