Differentiation of Nerve Terminals in the Crayfish Opener Muscle and Its Functional Significance

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ABSTRACT Junctional potentials (*jp*'s) recorded from superficial distal fibers of the crayfish opener muscle are up to 50 times larger than *jp*'s in superficial central fibers when the single motor axon that innervates the muscle is stimulated at a frequency of 1/sec or less. At 80/sec, in contrast, central *jp*'s are up to four times larger than those observed in distal fibers. The tension produced by single muscle fibers of either type is directly proportional to the integral of the time-voltage curve minus an excitation-contraction coupling threshold of 3 mv. Distal fibers therefore produce almost all the total muscle tension at low frequencies of stimulation and central fibers add an increasingly greater contribution as their nerve endings begin to facilitate in response to increased rate of motor discharge. Differentiation of muscle membrane characteristics (input resistance, space constant, time constant) cannot account for these differences in facilitation ratios. The mechanism of neuronal differentiation is not based upon the size or effectiveness of transmitter quanta, since equal sized *jp*'s have equal variances; *mjp* sizes and variances are also equal. No differences were found between fiber types in rates of transmitter mobilization, density of innervation, or the relationship between transmitter release and terminal depolarization. Single terminals on distal fibers were found to release transmitter with a greater probability than central terminals. More effective invasion of distal terminals by the nerve impulse at low frequencies can account for the difference.

INTRODUCTION

The capacity of crustacean muscles to give either fast or slow responses to indirect stimulation is now known to result largely from differentiation of the contractile properties of the muscle fibers themselves (1, 2, for a review see reference 3). Where a muscle is innervated by more than one motor axon, fast/slow differences may *also* depend upon the route by which excitation is delivered. Although early experiments emphasized possible differences in

neural properties (4–7), more recent investigations have shown that the different efferent nerves do not usually innervate the same population of muscle fibers. This report analyzes the role of nerve and muscle fiber differentiation in determining the relationship between neural excitation and tension production in the crayfish opener. The small size of this muscle makes it possible to measure tension development in single fibers from all its anatomical regions, as well as electrical activity resulting from stimulation of its motor axon.

The results show that different regions of the opener muscle contribute in different ways to the whole muscle tension at high and low firing frequencies of the single excitatory motor axon. It is clear, however, that this differentiation is based upon regional properties of nerve terminals and *not* upon those of the muscle fibers. The difference in quality of the responses depends upon dramatic differences in the facilitation rates of junctional potentials in several regions of the muscle. The muscle fibers themselves vary only slightly in such properties as effective input resistance, relative threshold for excitation-contraction coupling, and rate of tension increase in response to a given depolarization; these and other postjunctional variables cannot explain the regional differences in either junctional potential amplitude and facilitation rate, or tension development at a given frequency of stimulation.

METHODS

All experiments were performed on the cheliped opener muscle of the crayfish, *Procambarus clarkii*. Animals were collected locally or obtained from a distributor, maintained in aerated tap water tanks, and generally used within 3 wk. Autotomized chelipeds were pinned in a paraffin-filled Lucite dish in order to prevent movement of any segment. The preparation was maintained in van Harreveld's (8) solution at 20°–22°C; the fluid was periodically replaced.

The dorsal surface of the meropodite was chipped off with rongeurs, and the extensor and flexor muscles were removed in order to expose the nerve trunk more completely. The excitor nerve was always lateral to the inhibitor, and usually a naturally occurring cleavage plane existed between it and the bundle containing the inhibitor nerve. The separated nerve bundles were placed over platinum wire electrodes and independently stimulated with brief (0.2–0.4 msec) transformer-isolated square-wave pulses from one or two stimulators.

After the closer tendon to the dactylopodite was cut, the dorsal surface of the opener muscle was exposed in the propodite. Simultaneous intracellular recording from two to four muscle fibers was carried out using KCl-filled microelectrodes (resistance 7–20 MΩ) suspended from micromanipulators by fine silver wires. This "floating" arrangement eliminated some of the movement artifacts that often persisted after the opener tendon was fixed by pinning all claw segments. Signals were led through neutralized capacitance DC preamplifiers (Bioelectric Instruments, Inc., Hastings-on-Hudson, N. Y.), displayed on a multibeam oscilloscope, and photographed on moving film.

Larger KCl-filled microelectrodes (resistance 2–5 MΩ) were moved along the sur-

face of the muscle to record extracellularly from localized junctional areas while the excitatory axon was stimulated at frequencies of 1–5 Hz. Localized sources of extracellular junctional potentials were usually found after persistent probing (cf. 9).

An RCA 5734 transducer tube with a pair of fine forceps attached to the movable anode recorded tension. The opener tendon was cut and its proximal end was gripped near its insertion into the dactylopodite for whole muscle recording. For measuring tension from single or small clusters of muscle fibers with their nerve supply intact, a small piece of tendon with attached fibers was dissected free of the main tendon and gripped with the forceps. In order to avoid movement artifacts from nearby fibers and to avoid stretching the nerve innervating the isolated fiber bundle (cf. 2), the main tendon was fixed by pinning. Tension characteristics of proximal fibers could be obtained by cutting the central and distal fibers near their origins on the exoskeleton of the propodite and holding the tendon with the forceps. Such a procedure could not be used to isolate muscle fibers in other regions because the cutting of proximal fibers invariably blocked nerve conduction distal to the cut. The initial length of the muscle fibers was set by holding the dactylopodite at the half-open position, and contractions were almost entirely isometric for single fiber recording. More anode movement occurred during whole muscle recording, but the output of the transducer was nearly linear.

Two microelectrodes or more were inserted into a single fiber to pass current while measuring membrane voltage changes and/or tension. The current-passing electrodes were of 3–10 MΩ resistance and filled with 3 M KCl or 2 M K citrate; they were placed in series with a 75 MΩ resistor in order to provide constant current. Nevertheless, difficulty was still encountered in passing currents of more than 2×10^{-7} amp, especially when using KCl-filled electrodes (cf. 10). Current was measured across a 10 K resistor in parallel with a 10 μF capacitor between ground and one terminal of the stimulator. Other electrodes were used to record membrane voltage at various distances from the stimulating electrode. When tension was recorded from single muscle fibers, the stimulating electrode was placed in the middle of the fiber. This electrode placement gave almost uniform depolarization because of the long space constant. When such direct stimulation was applied, observation through a dissecting microscope verified that only a single fiber contracted. Recording electrodes in adjacent fibers never recorded significant membrane potential changes to current of either sign, further indicating that these fibers, like those of many other crustacean muscles, act as electrically independent units (10, 11).

The 20 ml of bathing solution could be completely replaced in 10–15 sec in experiments requiring changes in its ionic composition. Measurements were taken between 1 and 4 min after the solution had been changed in order to allow sufficient time for equilibration. Calculations of amplitude and variance of various parameters were carried out by programs written for IBM 7094 and SDS 940 computers.

RESULTS

Regional Differentiation of Junctional Potentials

The opener muscle is located in the next to last (propodite) segment of the claw. Individual muscle fibers arise from the dorsolateral aspects of the

propodite and insert in pinnate fashion on a central tendon which is attached to the dorsal, proximal surface of the dactylepodite. They all act synergistically to cause the dactylopodite to open.

The fibers form three to four layers and may be regionally divided into distal, central, and proximal fibers that may be further subdivided as superficial or deep. Fibers from each muscle region were examined.

A single excitatory axon innervates every muscle fiber, producing depolarizing junctional potentials (jp's) in all of them. When the axon was stimulated once every second, the amplitude of jp's in the superficial central (SC) fibers was often less than 0.1 mv and that in the superficial distal (SD) or superficial proximal (SP) fibers was usually greater than 0.4 mv. The difference was sometimes as great as 50:1 in a well-differentiated pair. In animals kept in the laboratory for less than 3 wk, 94 % (472/504) of fiber pairs recorded in different muscle regions were differentiated with respect to jp amplitude in the way described above; only 6 % (11/192) from the same region were so differentiated. On the rare occasions when two fibers in the same region were differentiated, the anomalous one was often accompanied by one or two adjacent fibers with similar characteristics. The probability of finding similar pairs in the same region was higher in animals kept longer than 3 wk; this occurred in 41 % of the cases (63/151 pairs in 20 animals). In four preparations, all fibers were of the same type in all regions. Since this breakdown in regional differentiation seemed to be associated with a deterioration in condition, the data that follow are all from animals kept less than 3 wk. No consistent differences were found with respect to time of year, time of moult cycle, sex, or size of claw (within a 7–22 mm range of propodite length).

All opener muscles contained about 300 fibers. Well-differentiated muscles usually possessed 15–25 SD fibers in the superficial distal $\frac{1}{5}$ to $\frac{1}{3}$ of the muscle's length, 35–55 SC fibers in the superficial central $\frac{2}{3}$, and 5–15 fibers of the SD type in the superficial proximal (SP) layer. The remaining 205–245 fibers were located in the deeper layers, and included SD and SC types scattered among fibers with intermediate characteristics.

After less than 4 sec of stimulation at low frequency (below 20 Hz) typical SD jp's were much larger than SC jp's; after 1 sec of high frequency (above Hz stimulation, SC jp's were larger than SD jp's (Figs. 1 and 2); at intermediate frequencies (20–60 Hz), a point of equality in SD and SC amplitudes occurred after 1–4 sec of constant frequency stimulation (Fig. 2). Frequency sweeps of 1–100 stimuli per sec taken every hour for 5 hr (Fig. 1 B) gave repeatable results. No changes in the properties of fibers were observed, even during the course of 14 hour experiments.

Recordings made from SD fibers during indirect stimulation appeared similar to those in the SP region, while those in the deeper layers distally (DD), proximally (DP), and centrally (DC) often showed properties that were

intermediate between SD and SC. Deep fibers in all regions also contained some very well-differentiated SD and SC types. The discontinuity between SD and SC fibers in the superficial layer usually occurred within two to four

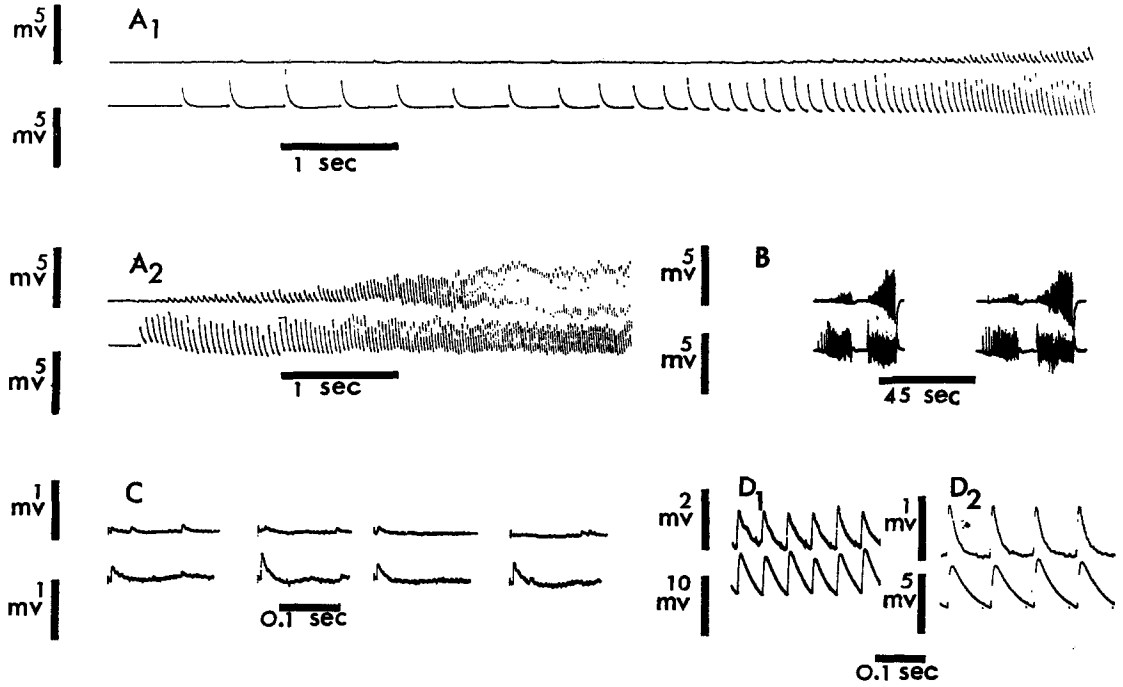

FIGURE 1. Simultaneous AC-coupled intracellular recordings from SC (top traces) and SD (bottom traces) fibers. A, frequency sweeps of 1–20 Hz in A_1 and 20–100 Hz in A_2. Input resistance, diameter, and time constants approximately equal for both fibers. Note that time constant decreases somewhat with increasing stimulus frequency. B, first (left) and last (right) samples of a series of 1–20, 20–100 Hz frequency sweeps take 1 hr apart for 5 hr. Fibers have an input resistance difference of 10-fold (SD > SC) and a diameter difference of 4-fold (SD < SC). C, single stimuli given once every 10 min. Note that SD jp's are larger than SC jp's, mjp amplitudes are about equal in the two fibers, and that no SC jp occurs in response to nerve stimulation in the first and last frame. SD input resistance seven times that of SC input resistance; fiber diameters about equal. D_1, SD and SC time constants about equal. Stimulus frequency, 20 Hz. D_2, SD time constant twice that of SC fiber. Stimulus frequency, 10 Hz.

cells. Fibers in this transitional zone were often intermediate between the two extremes.

Fig. 2 shows a typical example of a differentiated pair of fibers studied over a 5 hr period. Trains of 7000–10,000 stimuli at each of the frequencies shown were presented in random order, with 15 min rests between each train. Certain frequencies were repeated to show the reproducibility of results

(Fig. 2 C, E, F, G, H). Five pairs of differentiated fibers from five other animals studied in the same way gave qualitatively similar results.

In order to predict from the data of Fig. 2 whether a point of equal jp amplitude would occur in a pair of SD and SC fibers during a train of stimuli, one had to know both the frequency of stimulation and the number of stimuli that had been given. No jp's of equal amplitude were ever observed in well-

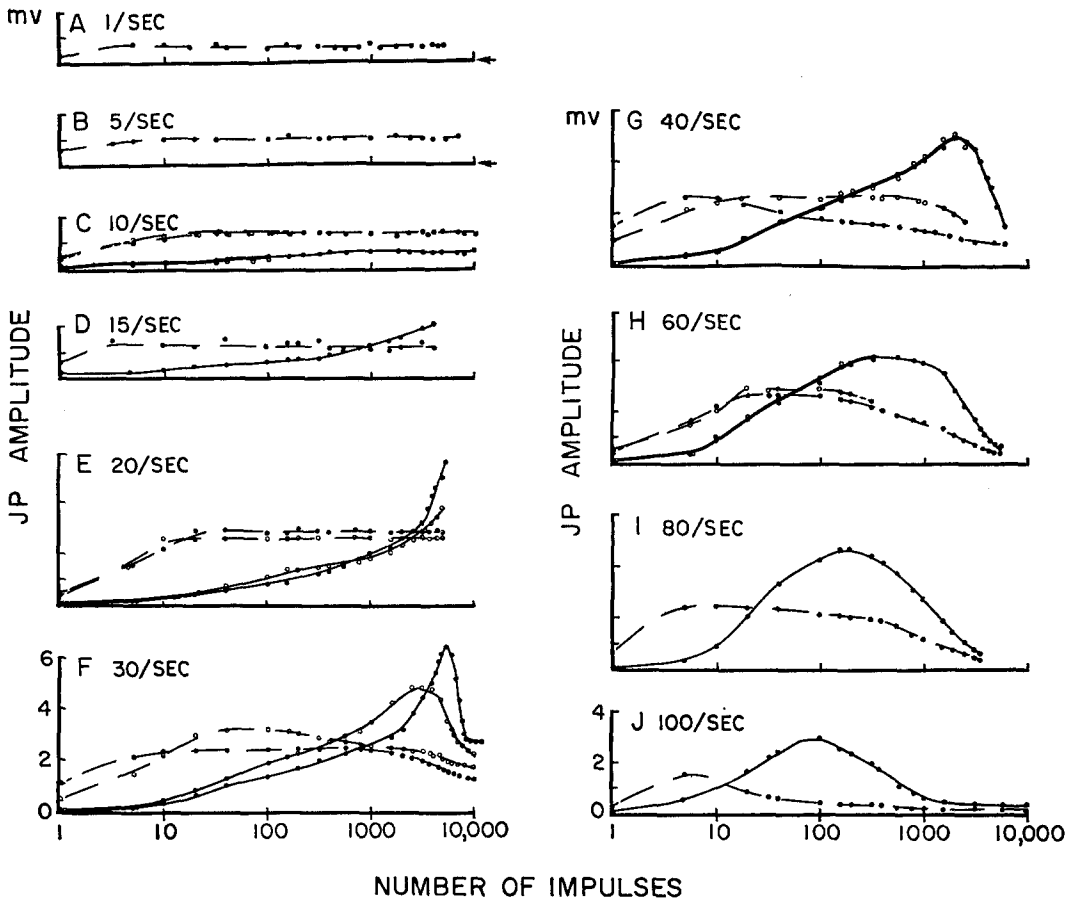

FIGURE 2. Differentiation of jp amplitudes with respect to number of impulses and frequency of stimulation recorded simultaneously from a single fiber pair. Ordinate, jp amplitude; abscissa, number of impulses given at the frequency shown for each insert. Each point averages 2–5 jp's at the appropriate time in the stimulus train. All scales equal to those shown in F and J. Dashed lines drawn through SD and jp amplitudes, solid lines through SC jp amplitudes. Filled circles, first time stimulus frequency presented. Open circles, second time that same frequency was presented. Arrows in A and B signify that jp amplitudes in SC fibers at those frequencies were about equal to mjp amplitudes (0.05 mv in both fibers) and hence too small to show up on the scale used. SD input resistance four times that of SC.

differentiated pairs of the two opposite types at frequencies less than 10 Hz. Jp's in SD fibers generally reached their maximum amplitude at any given frequency after 10–20 stimuli. The number of stimuli necessary for SC fibers to reach maximum amplitude depended much more on frequency than that for SD fibers, as described above. At 15 Hz, SC amplitude was often still increasing slowly after 10,000 stimuli; at 30 per sec, maximum amplitude was reached after several thousand stimuli; at 100 Hz, it was reached after 90 stimuli. With smaller numbers (<500) of equal interval stimuli, SC fibers attained their largest amplitudes at frequencies greater than 40 Hz. The amplitudes of SC and SD jp's declined after reaching maxima at frequencies of 30 Hz or greater. At 15 Hz, SD and SC jp's were often of equal amplitude after 1200 stimuli. At higher and higher frequencies, the point of equality was reached after fewer and fewer impulses, so that the crossover point occurred earlier.

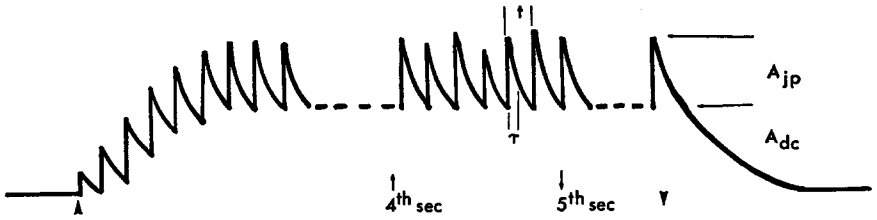

FIGURE 3. Method of calculating area under the membrane voltage vs. time records. Arrows indicate beginning and end of 4th second of stimulation. For explanation, see text.

The average depolarization level at any given time for any given frequency was affected by the membrane time constant, the jp amplitude, and the recent activity of the junctions on that muscle fiber. Fibers with jp's of equal amplitudes and time constants showed approximately equal amounts of depolarization. Occasionally this relationship did not hold (cf. 5), but the discrepancy could usually be accounted for by movement artifacts, which occurred despite the care taken to fix the opener tendon, or by damage to the muscle fiber. Plots of the DC depolarization level were obtained by calculating the area under the curve of membrane voltage vs. time between the 4th and 5th sec after beginning the stimulus train. This time interval was experimentally chosen because almost all fibers reached a DC "plateau" during the 3rd to 6th sec of stimulation.

The area under the curve was calculated by two methods. The first involved cutting out the photographic record between the 4th and 5th sec of stimulation, weighing it on a microbalance, and then comparing the result to weights obtained by cutting out 1 sec square wave depolarizations to various voltage levels. The second method (Fig. 3) involved measuring the average DC depolarization from the resting potential to the

base of the *jp*'s between the 4th and 5th sec of stimulation and adding it to the area under the *jp*'s. This latter area was calculated by assuming that each *jp* rose instantaneously and decayed exponentially until the next impulse occurred. Typical rise and decay times during 1 per sec stimulation were 4 and 30 msec, respectively. The time constant of decay was noted for each frequency (compare Fig. 1 D) and generally paralleled the decreased input resistance due to transmitter action. This phenomenon

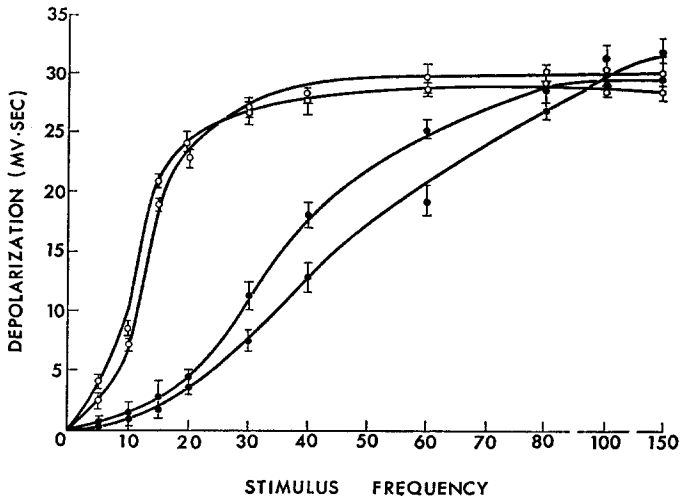

FIGURE 4. Average amount of depolarization (mv·sec, see text) developed at various stimulus frequencies applied to the nerve. Data taken from five well-differentiated SD (open circles) and SC (filled circles) fibers in each of two claws (separate lines drawn for each claw). Bars show one standard deviation in depolarization from the average for SD or SC fibers in a given claw at each frequency tested.

tended to limit the amount of depolarization which could be produced at high frequencies. The area under the entire curve is therefore given by the equation:

$$AV = A_{dc} + \frac{1}{t} A_{jp}\tau(1 - e^{-t/\tau})$$

where

AV = total area under the membrane voltage vs. time between the 4th and 5th sec of stimulation (mv-sec).
A_{dc} = DC amplitude between 4th and 5th sec (mv-sec).
A_{jp} = average *jp* amplitude (mv) (peak to peak).
τ = time constant of *jp* decay (sec).
t = interval between stimuli (sec).

Figs. 4 and 7 demonstrate that the average depolarization was greater in SD fibers at low frequencies. This is attributable to larger jp's in SD fibers rather than to their slightly longer time constants.

Mechanism Underlying Regional Differentiation

In order to determine whether the differentiation between SD and SC fiber types was due to the muscle fiber membranes, three separate approaches were used. In the first, the muscle fiber membrane was hyperpolarized 15–20 mv below its resting potential and held at that level in order to eliminate any

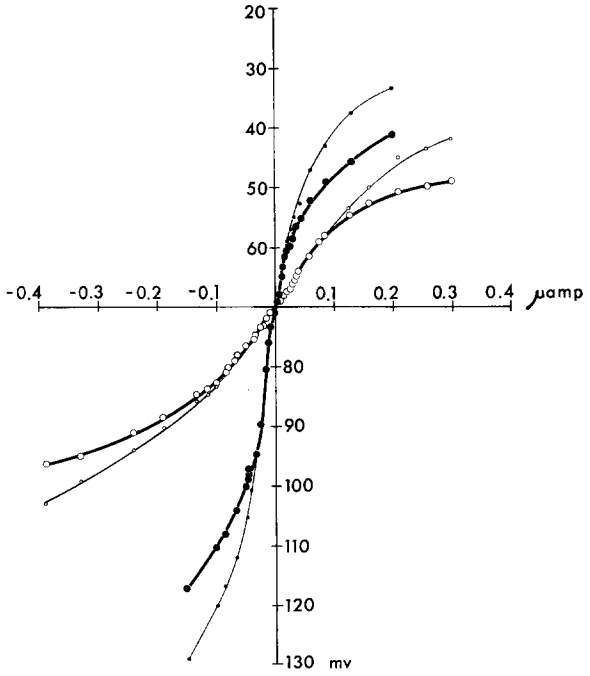

FIGURE 5. Current-voltage relation for typical SD (filled circles) and SC (open circles) fibers with delayed rectification. Abscissa, current in microamperes; negative current, depolarizing. Ordinate, absolute value of membrane potential in millivolts; resting potentials for both fibers, 70 mv. Heavy lines drawn through the final level of membrane potential; thin lines drawn through the height of the initial response. Both fibers are from the same claw, and are of equal diameter (200 μ) and length (3.1 mm). Note that the value of the input resistance (mv/μamp) depends upon the amplitude and sign of the current passed. At a membrane potential of 80 mv the SD fiber has a greater input resistance than at 70 mv or 100 mv.

voltage-dependent membrane responses. The nerve was then stimulated from 1–100 Hz. No change in the relative growth of SC or SD jp amplitude occurred with respect to time or stimulus frequency. Jp's in both fiber types were uniformly increased by 10–40 %, for two reasons: first, the input membrane resistance, as measured by the slope of the current-voltage relation, was often higher by 10–30 % at such hyperpolarized levels (Fig. 5); second,

the hyperpolarization presumably carried the membrane further from the equilibrium potential for the jp conductance change. Similar phenomena have been reported by Boistel and Fatt (12) and Reuben and Gainer (13) for other crayfish muscle fibers. Resting potentials for 59 distal, 45 proximal, and 79 central fibers averaged 66.5 ± 6.1, 67.6 ± 6.6, and 66.7 ± 6.2 mv SD, respectively, so that differences in that parameter could not account for the differences in jp amplitude.

The second method for estimating the role of the muscle membrane in differentiation involved determining current-voltage relationships for each fiber type. Figs. 5 and 6 show that both SD and SC fiber types responded only passively to prolonged current pulses; both often showed delayed rectification

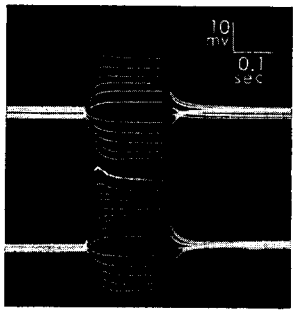

FIGURE 6. Membrane responses to current pulses of 200 msec in an SD fiber. Fiber length 4.5 mm, space constant 6.1 mm, diameter 300 μ, input resistance 2×10^5 Ω. Current-passing electrode near insertion on tendon. Electrode in upper trace is 0.05 mm from current-passing electrode; electrode in middle trace is 1.60 mm distant. The current pulses used to produce the simultaneously recorded potentials had values of 110, 80, 62, 53, 44, 36, 28, 21, 14, −7, −15, −24, −32, and −40 namp (top to bottom).

in response to currents producing hyperpolarizing or depolarizing voltage changes of more than 10 mv. A few fibers increased their resistance by up to 20 % if hyperpolarized or depolarized by 2–20 mv. Such input resistance changes, of course, cannot account for the amplitude changes of several thousand per cent which jp's underwent during nerve stimulation that produced similar ranges of depolarization (Figs. 1, 2, 4, and 7).

SD fibers had a higher average input resistance ($2.94 \times 10^5 \pm 1.55 \times 10^5$ Ω SD, for 113 fibers from 19 animals) than fibers of the SC type ($1.30 \times 10^5 \pm 0.85 \times 10^5$ Ω SD for 118 fibers from 18 animals). However, fibers of approximately equal input resistance were often well-differentiated (Figs. 1 A, 7 A, and B) and showed equal relative changes in resistance in response to equal amounts of indirect depolarization (Fig. 7). Since input resistance changes with polarization of the membrane (Fig. 5), its value was arbitrarily measured for hyperpolarizations of 8–12 mv in all experiments requiring comparison

of data between fibers. Because membranes were sensitive to damage by the microelectrode, especially in smaller fibers, data were taken only from fibers in which the average jp amplitude at a given frequency just after insertion of the electrode and just before its withdrawal did not differ by more than 20 %. Fibers of high input resistance tended to respond to 1 per sec nerve stimulation

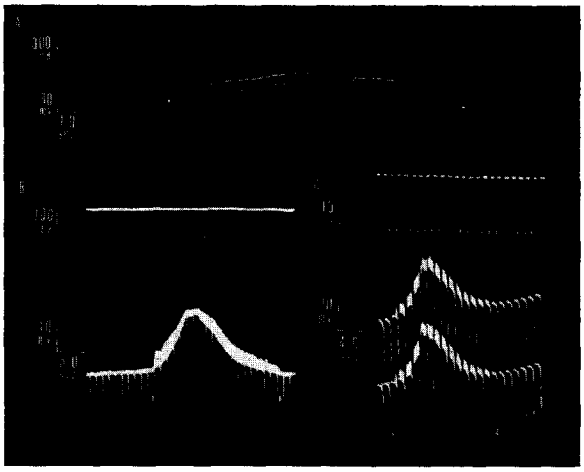

FIGURE 7. Changes in muscle input resistance produced by nerve stimulation. Top trace monitors current, lower trace(s) monitor membrane voltage. Nerve stimulation begins at upward arrow, ends at downward arrow. Fibers in A and B from same animal. A, SC fiber with 400 μ diameter, 3.5 mm length, 3.9 mm space constant, time constant 30 msec, input resistance 0.6 $\times$ 10^5 Ω. Middle trace 1.3 mm from current electrode, lower trace 0.1 mm. Frequency sweep of 10 $\rightarrow$ 100 $\rightarrow$ 10 Hz applied to nerve. B, SD fiber with 420 μ diameter, 3.5 mm length, 4.2 mm space constant, time constant 33 msec, input resistance 0.9 $\times$ 10^5 Ω. Lower trace 0.1 mm from current electrode. Frequency sweep of 10 $\rightarrow$ 50 $\rightarrow$ 10 Hz applied to nerve. C, SD fiber with 100 μ diameter, 2.1 mm length, 3.1 mm space constant, time constant 35 msec, input resistance 8.4 $\times$ 10^5 Ω at 10 mv hyperpolarization; input resistance 10.6 $\times$ 10^5 Ω at 10 mv depolarization by direct stimulation and 8.7 $\times$ 10^6 Ω at 25 mv depolarization; middle trace 0.7 mm from current electrode, lower trace 0.1 mm. Frequency sweep of 10 $\rightarrow$ 40 $\rightarrow$ 10 Hz applied to nerve. Note more rapid development of depolarization with frequency in SD fibers and large jp's at 10 Hz. SD jp's are antifaciliated on decreasing side of frequency sweep while SC are facilitated.

with large jp's. However, even the largest differences in input resistance observed in well-differentiated fiber pairs (about 10:1) could not account for the differences in jp amplitude at low frequencies (about 50:1). Furthermore, the amplitudes of spontaneous miniature junctional potentials (mjp's) were independent of input resistance. Consequently, measurements of input resistance in these and other crustacean muscle fibers may not be a measure of the properties of junctional membrane, as it is in frog twitch muscle (14).

The relationship between input resistance, jp amplitude, and developmental variables is discussed elsewhere.[1]

The third approach involved passing brief hyperpolarizing current pulses during stimulation of the excitatory axon to measure membrane resistance changes (Fig. 7). The membrane potential changes produced by constant current test pulses usually decreased monotonically to 30–70 % of the "resting" value as the stimulus frequency was increased from 0–100 Hz. A few SD or SC fibers showed a 20–30 % increase in input resistance during directly evoked depolarizations of 2–10 mv. Such changes could account for increases in test pulse amplitude of up to 20 % during indirect stimulation that produced the same amount of depolarization (cf. 13). In no case could these postsynaptic resistance changes account for more than a very small fraction of the jp amplitude changes that occurred over the same frequency range.

Jp's were often facilitated by as much as 4000 % in SC fibers during frequency sweeps between 1 and 60 Hz, and then were reduced by 1000 % between 60 and 100 Hz. Jp's in distal fibers were facilitated, usually by 300–600 %, from 1 to 40 Hz, and then were reduced by 3000–4000 % from 40 to 100 Hz (Figs. 1 and 2). As a control the peripheral inhibitor nerve to the opener was stimulated at 100 Hz. This always resulted in 30–70 % reductions in the membrane resistance (cf. 15).

These three experiments show that electrical properties of muscle fiber membranes cannot be responsible for the observed differences between central and distal fibers. The latter cannot be attributed to variation in the amount of summation due to different time constants of jp decay. Out of 114 well-differentiated fiber pairs, SD time constants were longer in 57, equal in 40, and shorter in 17. The placement of electrodes in relation to the location of terminals also cannot be the critical factor, since two electrodes placed anywhere in the same fiber always recorded any jp, mjp, or current pulse within 60 % of the same amplitude value (Figs. 6 and 7). This result is to be expected, since the membrane space constant is generally one to two times the fiber length. The innervation is thought to be broadly distributed over the fiber surface in this and other crustacean muscles (5, 9, 16), though many opener fibers seem to have their loci of innervation concentrated in the exoskeletal half of the fiber.[1]

Transmitter Release by Nerve Terminals

Since the properties of the muscle fiber membrane were shown not to be the cause of the central/distal differentiation, the ability of the nerve terminals to release transmitter was examined. The possibility that the density of

[1] Bittner, G. D. 1968. Differentiation of crayfish muscle fibers during development. *J. Exptl. Zool. In press.*

endings upon distal fibers was sufficiently greater to account entirely for their larger jp's in response to low frequency stimulation was ruled out by three independent procedures. First, the average number of branches to SC and SD fibers that can be supravitally stained with methylene blue was the same. Second, the average frequency of mjp's in 78 SC and SD fibers was 43.1 ± 20.3 and 41.4 ± 19.6 per min, respectively. Third, plots of the amplitude of SC and SD jp's against time or frequency were not parallel and indeed had slopes of different sign in some ranges (Figs. 1 A, 2, and 7). This would hardly be expected if the density of endings were the important factor in differentiation.

Single stimuli given once every 10 min (or even once per hour) were used to rule out differences in mobilization of transmitter as a cause of differentiation (Fig. 1 C). SD fibers had a 10- to 50-fold greater jp amplitude than SC fibers even under these conditions. Furthermore, mjp frequency during or after stimulation at various frequencies was not dramatically different in SC and SD fibers. During stimulation at 1–5 per sec, the average increase in mjp frequency (cf. 17) was 20 % more for SD than for SC fibers for 10 pairs studied—though some cases were observed in which a greater increase occurred in SC fibers. This 20 % difference in mjp frequency during nerve stimulation was probably the result of increased transmitter release at SD endings rather than its cause, since at no stimulus frequency was there a consistent correlation between the amount of facilitation and the increase in mjp frequency (cf. 17). In comparing the present results with those of Dudel and Kuffler (9, 15, 17), it should be borne in mind that they worked with the homologous muscles in the walking legs of a different species of crayfish.

In order to determine whether the size or effectiveness of transmitter quanta might be different in the two fiber types, the mean and variance of mjp and jp amplitudes were calculated for SD and SC fibers at various stimulus frequencies. If the release at single terminals is quantal in nature (9, 18) and if the membrane approximately sums the depolarizations produced at about 50 such terminals by each nerve impulse, one would predict that a greater quantal size or effectiveness would be correlated with a larger variance for the same average jp amplitude (cf. 18).

Fig. 8 is a plot of jp's produced by stimulation at 20 Hz or less. This value was selected because at higher frequencies jp's are attenuated due to temporal summation, which might cause their observed variance to differ from the true variance. Furthermore, at frequencies of 20 Hz or less, membrane conductance changes are always less than 15 % in either fiber type, and often less than 3 %. In order to get equal jp amplitude ranges for both types of fibers, data were taken at various times after the onset of stimulus trains, as in Fig. 2, or after a brief tetanus. Jp's or mjp's of equal amplitude always had equal variance at rest, or during increasing frequency of stimulation, or with

time at any given frequency. Therefore, as shown in Fig. 8, the quantal effectiveness remains constant between fiber types.

Fig. 9 plots jp's from five pairs of fibers in five equal sized claws, whereas Fig. 8 plots jp's from five pairs in the same claw. The scatter of variances at

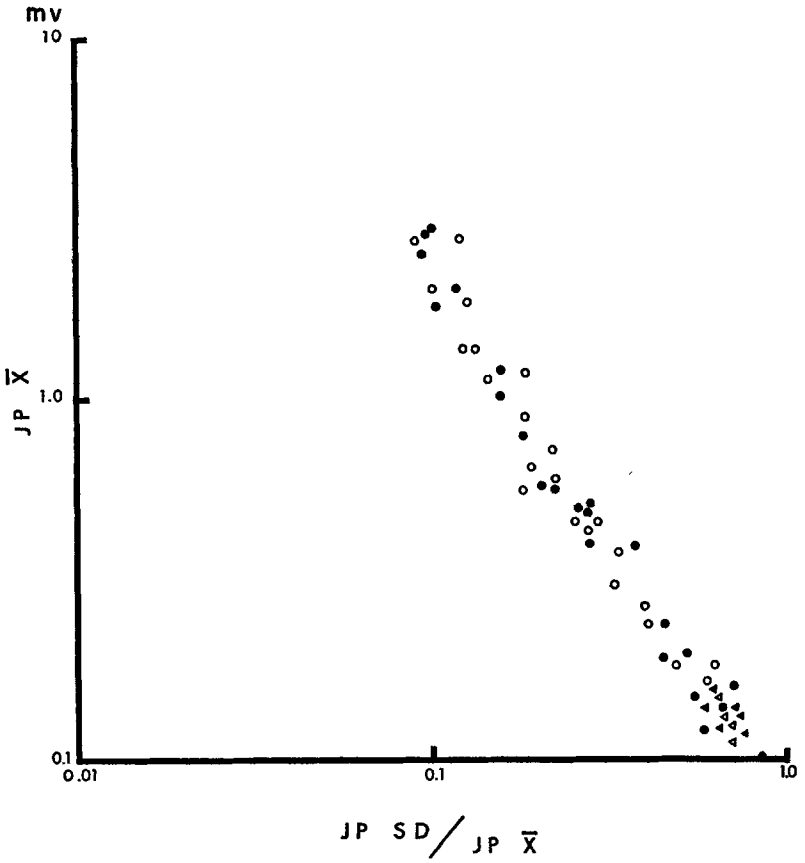

FIGURE 8. Mean jp amplitude (ordinate) plotted against normalized standard deviation; i.e., jp standard deviation/mean jp amplitude (abscissa). Data taken from five SD and five SC fibers in the same claw. SD jp's (open circles) and SC jp's (filled circles) produced by 1–20 Hz stimulation. SD mjp's (open triangles) and SC mjp's (filled triangles) taken at rest between stimulus trains are plotted on the same axes. Each point averages 20–40 jp's or mjp's.

any given amplitude is less in Fig. 8, indicating that the postjunctional effects of transmitter quanta are more uniform within a single individual than between individuals.

In order to test whether the relationship between terminal depolarization and transmitter release might differ in SD and SC fibers, the KCl concentration in the van Harreveld's solution bathing the preparation was doubled

while NaCl was reduced in order to keep Cl^- concentration and osmotic pressure constant. The change in mjp frequency before and after changing the K^+ concentration was recorded in three well-differentiated fiber pairs in each of three animals (Table I). Mjp frequency increased 84% in SD fibers and 95% in SC fibers; the difference between these values is not statistically

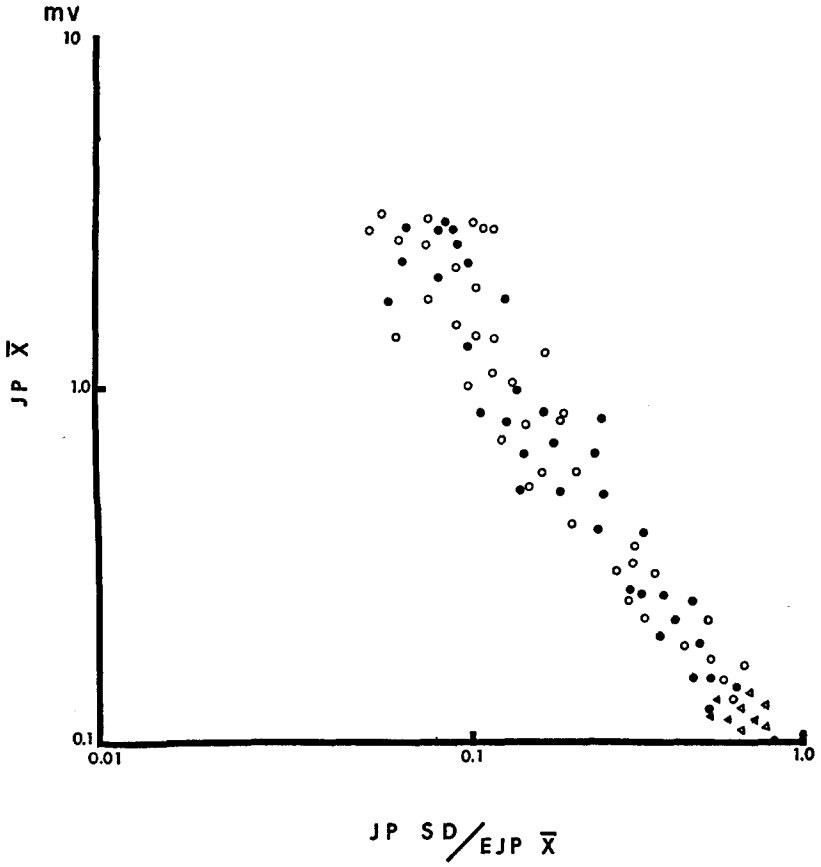

FIGURE 9. Mean jp amplitude (ordinate) and normalized standard deviation (abscissa) Jp's taken from one SD (open circles) and one SC (filled circles) fiber from each of five animals during 1–20 Hz nerve stimulation. SD mjp's (open triangles) and SC mjp's (filled triangles) also plotted on the same axes. Each point averages 20–40 jp's or mjp's.

significant. The amount of muscle fiber depolarization observed was about 7 mv in each case (cf. 9) and only slight changes in average mjp amplitude or muscle membrane conductance occurred. The size of evoked jp's at 5–10 per sec usually decreased in both fiber types during exposure to the high K^+ solution, and returned to normal within 1 min after the return-to-normal van Harreveld's solution. Larger increases in K^+ concentration produced qualitatively similar results.

Extracellular recordings of localized junctional potentials were made using fibers from well-differentiated SC and SD regions of the muscle. The results are given in Table II. The probability of quantal release at low frequencies of stimulation was much greater in distal than in central fiber terminals. As the frequency of nerve stimulation was increased from 1–10 per sec, the probability of release at single central fiber endings increased more than that at single distal endings (390:80%). This difference was accompanied by a comparably greater increase in jp amplitude (460:80%). The agreement between E_1 (size of quantum) and E_s (average spontaneous potential) in each

TABLE I

CHANGE IN mjp FREQUENCY ASSOCIATED WITH INCREASED EXTRACELLULAR POTASSIUM

		A		B	
Animal No.	Fiber pair No.	SD	SC	SD	SC
				%	%
1	1	31	47	+40	+80
	2	52	22	+65	+92
	3	43	34	+89	+67
2	1	33	32	+72	+87
	2	61	46	+115	+121
	3	19	37	+108	+92
3	1	12	13	+130	+120
	2	8	11	+58	+92
	3	19	14	+79	+110

Column A, average mjp frequency per min in an SD and SC fiber pair (recorded simultaneously) before increasing extracellular potassium by two-fold.

Column B, Average % increase in mjp frequency after increasing extracellular potassium concentration. Three 1 min periods averaged for each fiber pair.

case was in the range reported for opener muscle terminals (9, 15, 19, 20) and so were calculated values of m (average number of quanta released per impulse). The correlation between facilitation of intracellular jp's (E_r) and m for most endings in the opener muscle was studied by Dudel (20) and Dudel and Kuffler (17), but they did not note the muscle regions from which they took their data. Experiments 1–3 of their Table 1 (page 536) (17) would appear to have employed SC fiber types as would Nos. 3, 5, 7, 9, and 15 of Table 2 (pages 328–329) (29); Nos. 1, 2, and 11 of Table 2 (pages 328–329) (20) would seem to be SD types. Since the mechanism for this increased probability of transmitter release has been shown *not* to involve mobilization of transmitter, or differences in the relation between transmitter release and

amount of terminal depolarization, differences in the effectiveness of terminal invasion by nerve impulses remain as the most likely possibility.

Differentiation of Tension Production

The functional significance of this neuronal differentiation was examined by stimulating the excitor nerves of several animals at gradually increasing

TABLE II

CORRELATION BETWEEN FIBER TYPE, INTRACELLULAR jp AMPLITUDE, AND PROBABILITY

Animal fiber		m			$E_1(\mu v) - E_s(\mu v)$	E_I		
No.	Type	1/sec	5/sec	10/sec		1/sec	5/sec	10/sec
						mv	*mv*	*mv*
1	SC	0.22	0.34	0.49	82-71	0.17	0.30	0.75
	SD	1.50	2.30	2.60	68-67	1.50	2.00	2.50
2	SD 1	3.10	2.70	3.90	40-47	1.80	2.50	3.50
	SD 2	2.30	3.10	2.90	47-49	2.80	3.30	3.30
3	SC 1	0.18	0.45	0.79	62-60	0.13	0.46	0.84
	SC 2	0.08	0.36	0.51	89-80	0.06	0.28	0.65
	SD	1.20	1.90	2.70	77-82	0.73	2.10	3.00
4	SC 1	0.33	0.29	1.30	54-70	0.13	0.48	1.10
	SD 2	2.30	2.20	2.90	55-54	2.20	2.40	2.50
5	SD	1.20	2.30	3.60	63-55	1.50	2.10	2.30
	SC	0.05	0.11	0.33	38-44	0.11	0.37	0.69

m = average quantum content of extracellular jp's calculated from $m = \log_e n/n_o$, in which
n = number of stimuli (100 in each case), and
n_o = number of transmission failures.

E_1 = size of extracellular quantum determined from $E_1 = \bar{E}/m$, in which
$\bar{E}$ = average size of extracellular jp, and
E_s = average size of extracellular mjp's.

E_I = average intracellular jp amplitude recorded in the same or nearby fibers.

frequencies from 0–60 Hz, while observing the entire opener muscle through a dissecting microscope. At frequencies of 5–10 Hz the SD and SP fibers could be seen to shorten. SC fibers did not contract enough to take up the slack created in them by the action of SD and SP fibers on the common tendon of insertion until the frequency reached 15–25 Hz. Single muscle fibers or groups of two to four fibers were then isolated from the common tendon and the tension they exerted in response to direct and indirect stimulation was recorded with a mechanoelectric transducer.

Fig. 10 indicates that the tension developed by the entire muscle as a function of stimulus frequency is a composite of the frequency-dependent tension exerted by each of the differentiated regions. The tension was plotted

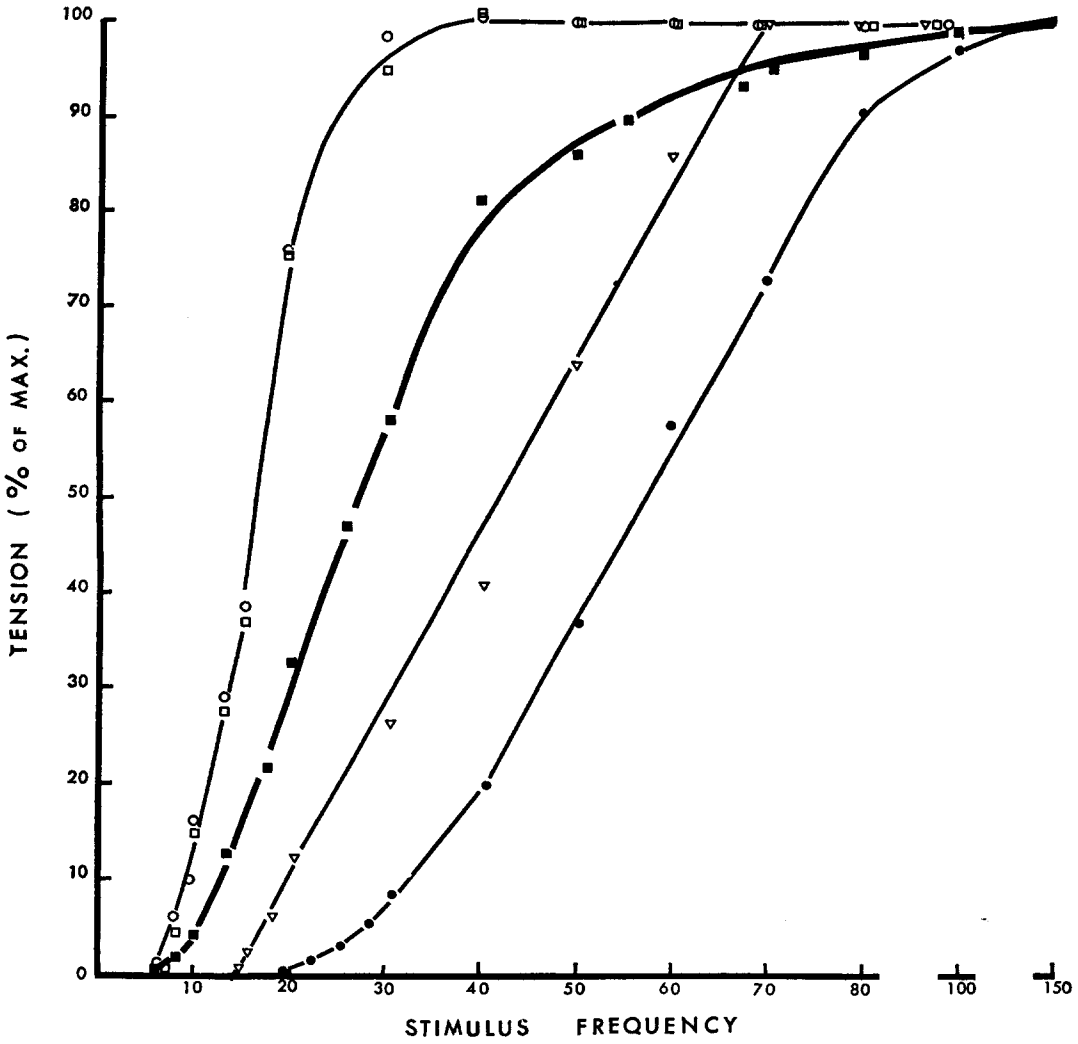

FIGURE 10. Per cent of maximum tension developed by a single SD fiber (open circles), SP fiber (open squares), deep central fiber (open triangles), SC fiber (filled circles), and from the entire muscle (filled squares, heavy line) recorded from a single claw as a function of stimulus frequency.

as a percentage of the maximum tension produced by each fiber, in order to eliminate differences in absolute tension due to fiber diameter or orientation. As the frequency increased, first the SP and SD fibers contracted, then many fibers in the deeper layer were activated, and finally SC fibers shortened.

Maximum whole muscle tension was not reached until the SC fibers added their final increment at frequencies of 100 per sec or more. The maximum tension produced by a single fiber was usually equal to 1 per 100-1 per 500 of the maximal whole muscle tension; the maximal amount of tension de-

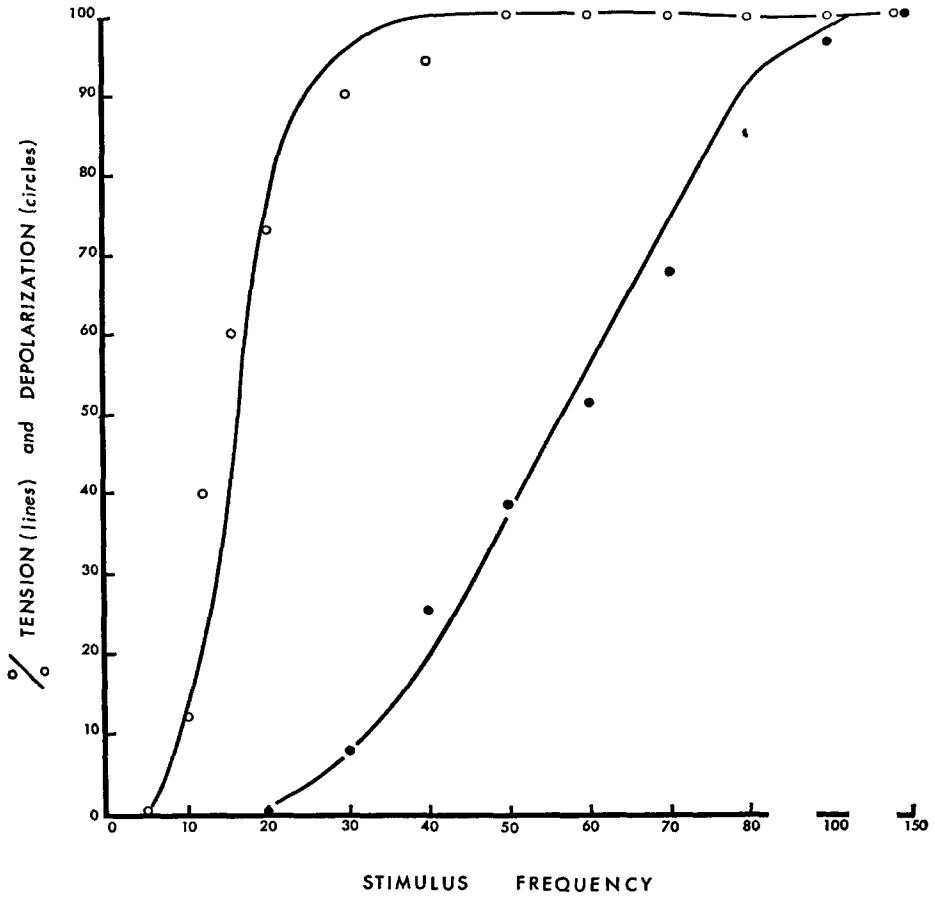

FIGURE 11. Per cent of maximum depolarization developed by an SD (open circles) and an SC fiber (filled circles) as a function of stimulus frequency. SD maximum depolarization = 28 mv, SC = 29 mv. Solid lines, per cent of maximum tension developed by the same fibers at various frequencies. Same SD and SC fibers as in Fig. 10.

veloped by a single fiber was proportional to its cross-sectional area. Since the mean cross-sectional area of SC fibers is 1.2- to 1.4-fold greater than that for SD fibers, an average SC fiber probably contributes slightly more to whole muscle tension than the average SD fiber.

Tension development in single SC and SD fibers measured in response to current passed through a second intracellular electrode was found to be

directly proportional to the amount of depolarization. Fig. 11 was obtained from the same pair of SC and SD fibers shown in Fig. 10, each of 150 μ diameter and 10 μ sarcomere length, by plotting per cent maximum tension development (solid lines) and per cent maximum depolarization above resting potential (circles) vs. stimulus frequency. Maximum depolarization

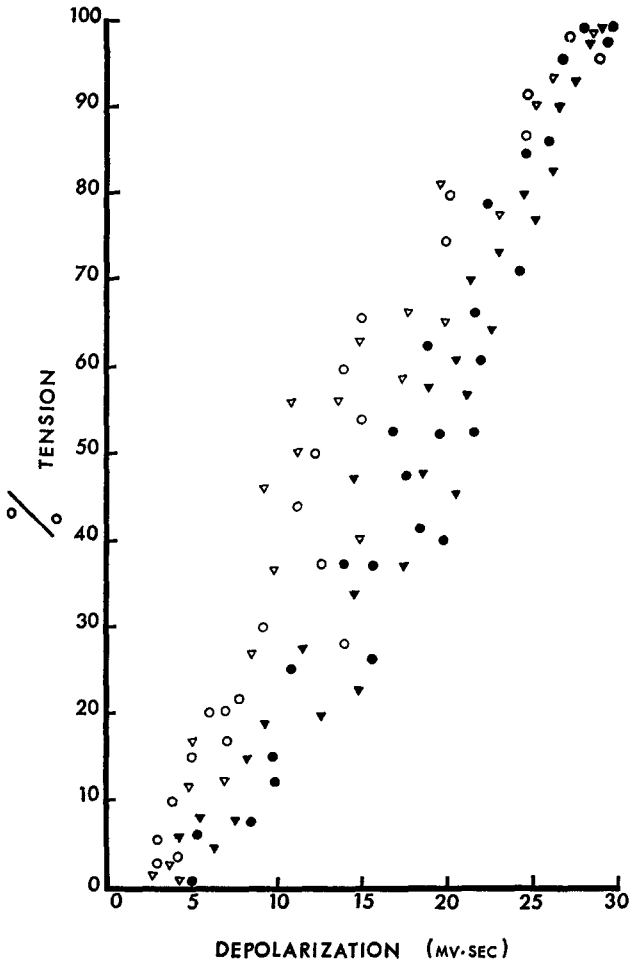

FIGURE 12. Per cent maximum depolarization (ordinate) vs. average amount of depolarization between the 4th and 5th sec of stimulation (see text). Direct stimulation in SD (open triangles) and SC (open circles) fibers plotted on the same axes used for recording responses to indirect stimuli in SD (filled triangles) and SC (filled circles) fibers. Data taken from 10 fibers with a diameter range of 60–400 μ from five claws of 8–21 mm propodite length. Maximum tension in response to indirect stimulation is defined as the tension produced by 150 Hz stimulation, which usually produced between 20–30 mv depolarization; maximum tension in response to direct stimulation is defined as the maximum tension produced by depolarizations of 25–30 mv in each fiber.

was taken to be 30 mv, since this was about the value of the plateau attainable by indirect stimulation. The points from the two fiber types lay on approximately the same curves when plotted on the same axes, indicating that the amount of tension produced by each was determined exclusively by the amount of membrane depolarization. The relationship between membrane depolarization and tension production between the 4th and 5th sec of stimulation was shown to be the same for both fiber types. In general, equal amounts of depolarization produced equal amounts of tension as a per cent of maximum tension produced in both fiber types. Slightly greater tension development occurred with direct depolarizations of less than 15 mv than with neurally evoked depolarizations to the same level (Fig. 12). In all cases tension increased slowly (over a period of several seconds) during depolarization. With depolarizations which have a rapid rise time, such as those produced by high frequency nerve stimulation or DC current pulses, the excitation-contraction coupling mechanism responds to the total depolarization within the 5 sec period. But with low frequency nerve stimulation the depolarization itself has a rise time of several seconds, so that the tension-producing mechanism reacts to a gradually increasing depolarization rather than to one that is constantly high for the time interval involved. This accounts for the smaller tension development with low frequency stimulation seen in Fig. 12. The responses of most fibers to indirect stimulation showed the following features commonly found in other crustacean tonic fibers (2, 3, 10): (*a*) Tension continued to develop very slowly for a period of many seconds during depolarizations of 3–15 mv; (*b*) tension continued to develop for 100–400 msec after membrane depolarizations of 10–15 mv or greater were discontinued; (*c*) relaxation after a near maximum contraction required 0.5–1.0 sec.

Histological examination of both muscle fiber types revealed no differences in sarcomere length (8–10 μ) or myofibril clumping in propodites 8–26 mm in length.[1] These observations are consistent with the slow rate of tension development observed in all fibers (cf. 2, 3).

DISCUSSION

Comparisons with Other Crustacean Muscles

The results show that nerve terminals of the single excitatory axon innervating various regions of the crayfish opener muscle are reliably differentiated for transmitter release. This neuronal differentiation is functionally significant in terms of tension development by single fibers and by the opener muscle as a whole.

Kennedy and Takeda (21) proposed that differences in junctional potential (jp) amplitude and facilitation ratios in fibers from the superficial flexor

muscles of the crayfish abdomen might be due to differences between properties of axon terminals. Irvani, in a brief note (22), described larger jp's in distal, deep, and proximal fibers than in central superficial fibers of the crayfish opener muscle during 15 per sec nerve stimulation. Atwood and Hoyle (23) reported different apparent rates of facilitation in response to stimulation of the fast axon innervating the closer of the crab, *Randallia ornata*. None of these reports, however, eliminated the possibility that muscle fiber differentiation, which has been found to be the cause of commonly observed neurophysiological and behavioral phenomena in crustacean muscles (1–3, 25) was involved. Fibers in many crustacean limb muscles exhibit a continuous spectrum of properties with respect to membrane excitation and tension production. The extremes of this spectrum can be conveniently identified by rates of contraction in response to membrane depolarization. Table III is a summary of these and other properties, comparing them with those of the opener muscle. In general, the characteristics of the opener muscle fibers agree with those reported by others for slow (tonic) crustacean fibers, though the opener fibers are intermediate in membrane time constant and diameter. Average fiber diameter is proportional to claw size in the crayfish opener; since no mention of this developmental variable is made by other authors, precise comparisons are difficult. Differentiation like that described in Table III does occur in other muscles that—like the opener—are supplied by a single excitatory axon (2, 24). Such subpopulations of muscle fibers, perhaps differing in input resistances and density of innervation, have usually been suspected when differences in jp amplitudes are encountered in and between fiber types.

Postsynaptic Characteristics

Differences in input resistance do exist between superficial distal (SD) and superficial central (SC) fiber types, $2.94 \pm 1.55 \times 10^5$ Ω SD to $1.30 \pm 0.85 \times 10^5$ Ω SD, respectively. However, this difference cannot account for the 10- to 50-fold differences in the amplitude of jp's evoked by single stimuli. SD and SC fibers with equal input resistances may be well-differentiated with respect to jp amplitude. Within either class there is a slight correlation between jp amplitude in response to low frequency stimulation and input resistance, but equal amplitude jp's have equal variances in such fibers. The fact that the amplitude and variance of spontaneous miniature junctional potentials (mjp's) are independent of membrane input resistances, also indicates that input resistance does not determine jp amplitude. If mjp's are presumed to result from the release of single transmitter quanta, then measurements of membrane input resistances do not indicate the relative effectiveness of those quanta as they do in vertebrate muscles (cf. 14). Atwood (25) has suggested that in tonic fibers of the crab closer muscle differences in facilitation rate as a

TABLE III

COMPARISON OF PROPERTIES OFTEN ASSOCIATED WITH PHASIC OR TONIC FIBERS AND THOSE OF THE CRAYFISH OPENER

Property	Phasic fibers	Tonic fibers	Crayfish opener fibers
1. Time to plateau tension in response to a depolarization of more than 20 mv (*msec*)	10–60	500–1500	300–1000
2. Tension maintained during a long lasting depolarization	No	Yes	Yes
3. Response to brief (<10 msec) depolarizations of more than 20 mv	Twitch	None	None
4. Structure (cross-section)	Dispersed myofibrils	Clumped myofibrils; central core	Clumped myofibrils; central core
5. Sarcomere length, μ	2–7	7.5–14	8–12
6. Fiber diameter, μ	300–800	50–150	100–450*
7. Length constant, *mm*	0.5–2	1.5–6.10	1.5–9.0*
8. Time constant, *msec*	5–50	30–800	20–90*, ‡
9. Input resistance, Ω	1–3 × 10^4	10–40 × 10^4	3–100 × 10^4 *, ‡
10. Specific membrane resistance, Ω cm^2	200–600	1000–20,000	1000–25,000*, ‡
11. Membrane responses to indirect stimulation	Large spikes, graded responses: common; passively responding fiber, delayed rectification: rare	Large spikes, graded responses: rare; passively responding fibers, delayed rectification: common	Large spikes, graded responses: never seen; passively responding fibers: uniform; delayed rectification: common

* Parameter shown to be dependent on propodite length.

‡ Parameter shown to be dependent on fiber type within the opener muscle.

Data compiled from Atwood (1, 3, 25), Atwood et al. (2), Atwood and Dorai Raj (24), and the present study.

function of stimulus frequency may be accounted for by the amount of change in input resistance as a result of transmitter action. Such differences do not occur among opener muscle fibers, as was shown by measurements of input resistance during nerve stimulation. Furthermore, since jp variances are equal in the two fiber types for equal jp amplitudes independent of the frequency of stimulation from 0–20 per sec, the postsynaptic effect of the transmitter must be the same in both fiber types. Thus, the two fiber types cannot differ dramatically in the effective input resistance of their junctional membranes.

Since the observed differentiation does not lie in the postsynaptic membrane, it must lie in the properties of the nerve endings upon the two fiber types.

Characterization of Neuronal Differentiation

In order to compare the development of depolarization in SD and SC fiber types, one needs to know more than the stimulus frequency, since jp amplitude also depends on the number of previous stimuli. For example, the amplitude of jp's in fibers from different muscle regions responding to a stimulus frequency of 15 per sec (22) can be shown to depend on the time at which they are recorded during a constant frequency stimulus. The ratio of amplitudes between SD and SC fibers depends upon stimulus frequency: below 20 per sec, it is greater than one; above 50 per sec, it is less than one. Furthermore, preliminary studies have shown that the stimulus pattern has much more dramatic effect on SC jp amplitude, and that conditioning tetani at 100 per sec affect the responses of SC fibers to 1 per sec test stimuli more than those of SD fibers.

Mechanisms of Neuronal Differentiation

Neuronal differentiation cannot be attributed solely to differences in the density of innervation since no differentiation occurs in postsynaptic junctional membrane, and plots of jp amplitude and frequency for the two fiber types are not parallel. The facts that average mjp frequency and the number of vitally stained nerve branches are identical in the two fiber types also implies that innervation density is not the critical factor. The fact that equal sized jp's have equal variance in any fiber implies either (*a*) that quantal size and the effective input resistance of junctional membrane are equal in the two fiber types, or (*b*) that quantal size or receptor density and the input resistance of junctional membrane vary inversely to keep quantal effectiveness constant. Differences in the rate of transmitter mobilization could easily be ruled out as a significant factor, since single stimuli given only once every 10 min still produced large SD jp's and small SC jp's. Furthermore, the increase in mjp frequency occurring during low frequency stimulation (9) is about the same for both fiber types.

All the preceding observations agree with the results of extracellular recording from single terminals, where the probability of release in response to stimuli at 1 per sec is much greater in distal than in central fibers. The increase in probability of release with increasing frequency of stimulation is much greater in central fibers, paralleling the changes in size of intracellularly recorded jp amplitude. This increased probability of release cannot be accounted for by differences in the slope of the relationship between probability of transmitter release and amount of depolarization, since changes in extracellular potassium concentration produce approximately equal changes of mjp frequency in both fiber types (cf. 26). While questions exist as to whether the increased extracellular potassium may have effects on transmitter release other than that of depolarizing the presynaptic terminals (27), the fact that both fiber types respond similarly rules out any important differences in this variable.

The remaining explanation for the differing probabilities of release at single nerve endings involves differing amounts of terminal invasion by the nerve action potential. (An alternative—equal amounts of terminal invasion by an active membrane process, accompanied by different extracellular ionic concentrations which produce action potentials of different sizes—can probably be ruled out in view of the electrotonic nature of the potentials in terminals of the homologous walking legs (19).) Low frequency stimulation might be accompanied by a greater passive change in the membrane potentials of SD terminals, resulting in a greater probability of transmitter release than exists at SC terminals. As frequency increases, the potential change in SC terminals might increase, possibly due to longer lasting hyperpolarization. Increases in the amplitude of terminal potentials recorded from unspecified regions of the opener muscle have been correlated with increasing frequency of stimulation and increasing probabilities of transmitter release (Table II, 28, 29). The mechanism for the decrease in jp amplitude observed during high frequency stimulation has not been specifically studied, but its extent is much greater than the postsynaptic changes in membrane resistance. The antifacilitation is very brief, since test stimuli given 300 msec after conditioning trains of 100 per sec stimuli sufficiently long to cause antifacilitation are at least equal to control values in SD fibers and are potentiated up to 2000% in SC fibers.

After this paper was written, Atwood (28) briefly reported finding "facilitating" and "nonfacilitating" jp's in different fibers of a crab opener muscle which appear to be similar to the jp's from SC and SD fibers, respectively, described in the present report. Extracellular recording at low frequency stimulation from single nonfacilitating endings revealed higher probabilities of transmitter release than from facilitating endings (as in the crayfish opener); nonfacilitating endings also had larger terminal potentials. Extracellular

recordings from crayfish SD fibers (Table II) also revealed larger terminal potentials than from most SC fibers, but since terminal potential is highly dependent on electrode position (19), it would have been necessary to sample many more endings to demonstrate a statistically significant difference. These observations support the notion that more complete terminal invasion by the axon spike occurs in SD (nonfacilitating) than in SC (facilitating) nerve terminals at low frequencies.

Functional Significance of Neuronal Differentiation

Equal amounts of depolarization produce equal amounts of tension in the two fiber types. Therefore, the differentiation in jp amplitude between fiber types is directly reflected in the amount of tension development: only SD fiber types contract at low frequencies of stimulation, while SC fiber types add their complement of tension at higher frequencies, where SD tension development is already maximal. The greatest level of depolarization is no more than 20–30 mv; excitation-contraction coupling occurs at 3–4 mv below the resting potential, no matter what its level from 52–80 mv. Therefore, these fibers all act like the "supersensitive" fibers reported for other crustacean muscles (2, 24).

Since equal amounts of depolarization produce equal amounts of tension, independent of whether direct or indirect stimuli are used, nerve terminals with a special relationship to the excitation-contraction coupling mechanism cannot exist. The good correspondence between jp amplitude and tension production gives further indication that jp differentiation cannot result from differences in postsynaptic properties, as suggested for the hermit crab opener by Wiersma and Bobbert (7).

In summary, the opener muscle of the crayfish claw is anatomically a single motor unit, with muscle fibers that are functionally similar in terms of their excitation-contraction coupling mechanism. However, the nerve terminals on these fibers are not all alike; instead, they are differentiated so that an increasing number of fibers contribute tension as the stimulus frequency is increased. This differentiation of the nerve terminals allows for fine tension gradation over a much wider frequency range than would be possible if only SC or SD types were present. If the muscle consisted entirely of SD fiber types, tension could be finely graded only over a range of rather low frequencies; if it were composed of SC types, no tension at all could be produced at low frequencies. Furthermore, the rate of tension development is slightly greater in SD type fibers at middle frequencies because of the more rapid facilitation found in these fibers; since SC fibers show less rapid antifacilitation, they maintain maximum tension for a longer time at middle and high frequency ranges.

Atwood et al. (2) propose that differentiation of crustacean muscle fibers

increases the range of tension control peripherally in a nervous system which seems to have very few nerves available to innervate its muscles. Differentiation of nerve terminals would also provide additional peripheral control of whole muscle tension. The relative differences in facilitation ratios reported (2, 23, 25) for other crustacean muscles whose major functional differentiation occurs in their muscle fibers, may indicate that neuronal differentiation plays a minor role in their whole muscle tension gradation. By contrast, it plays a major role in the opener muscle.

A similar differentiation of the terminals of a single neuron with respect to temporal variations in transmitter release may be important in other neural systems as well. The excitatory axon to the opener muscle innervates an average of 300 opener muscle fibers in addition to about 1000 stretcher fibers. If each of the stretcher fibers bears approximately 50 terminals, as do those in the walking-leg opener (9 and footnote 1), then this nerve has a total of about 65,000 output synapses—a figure that might reasonably be applied to a complex neuron in a vertebrate central nervous system. In a portion of central nervous system where information is being collected from a two-dimensional array of receptors, regional differences in temporal characteristics of the synapses made by a single neuron could account for some familiar phenomena. Suppose, for example, that the 65,000 output terminals of an interneuron in the visual system were represented as occupying a circular region, with that neuron's soma in the middle corresponding to the center of a retinal receptive field. If, as is the case with the opener axon, "central" junctions increased in efficacy with repeated high frequency stimulation while distal ones decreased, activity at the next level would tend to be focused upon an increasingly narrow core of postsynaptic elements. The corresponding receptive fields recorded in these postsynaptic neurons should thus change to a more punctate form having lower convergence; just this phenomenon has, in fact, been demonstrated in the cat's visual system by Barlow et al. (29), who showed that ganglion cell receptive fields shrink in size during light adaptation.

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