THE EXCITABLE PROPERTIES OF THREE TYPES OF MOTOR AXONS

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In previous descriptions of the three types of motor axons found in the walking legs of crustacea, it was suggested that these fibers have quite dissimilar excitable characteristics (Wright and Adelman, 1954; Wright and Coleman, 1954). In the experimental work reported here, there were many distinct differences in procedures with respect to the earlier work. Also, the data obtained by Wright and Adelman were from the crayfish, *Cambarus bartoni*. The present work on the normal excitable properties of lobster axons was undertaken to seek further evidence for the aforementioned contention, and to extend our information about the excitable process itself.

Methods

Opener, slow closer, and fast closer motor fibers from the limbs of the lobster, *Homarus americanus*, were studied by the method of Wright, Coleman, and Adelman (1955). Stimulation and recording techniques were as previously described (Adelman, 1956 *a*). Essentially, all responses were initiated and recorded at an oil-saline interface, and may be considered as derived from axons immersed in physiological solution. Cole's *Homarus* solution was used throughout the study (W. Cole, 1941).

RESULTS

(*a*) *Responses to Constant Current Stimulation.*—One of the most striking properties of these axons was their ability to respond with more than one action potential when stimulated with long duration currents of slightly greater than rheobasic strength. This property of repetitive discharge was present in all three types of fibers investigated. Occasionally, a non-repetitive fast closer axon was prepared, but this was more often the exception than the rule. For the most part, a given voltage determined a sequence of impulses for a given duration of current. In fast closer fibers, this total number of elicited spikes was usually less than the total number of spikes elicited by

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comparable voltages (in terms of rheobase multiples) from either openers or slow closers.

Upon constant current stimulation, as the strength was increased beyond the voltage which gave a long train of spikes, a number of the spikes began to "drop out" of the sequence. This phenomenon usually resulted in low

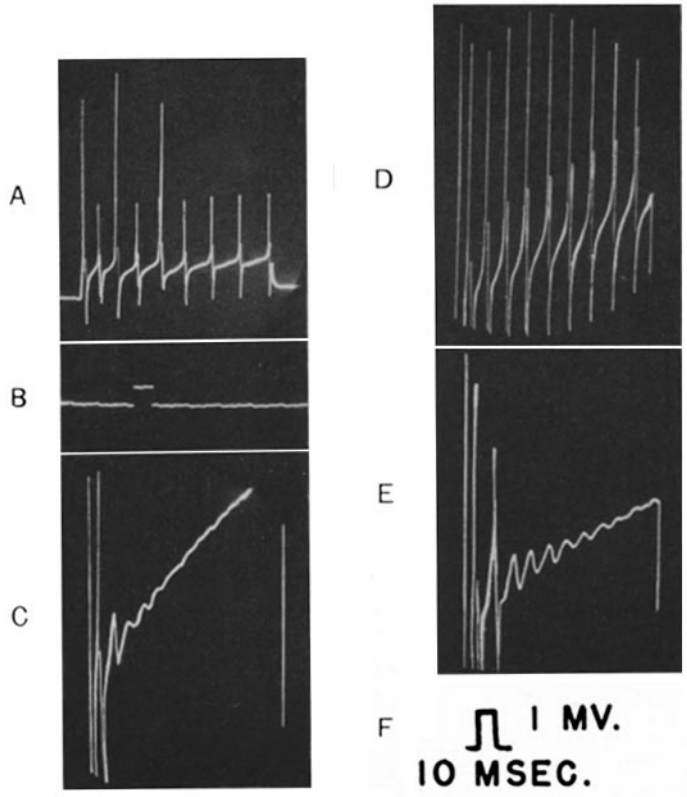

FIG. 1. Repetitive responses obtained from a twin fiber preparation (fast and slow closers). Cathodal depression. (See text.)

amplitude potentials appearing in the places where the spikes had been. This cathodal depression of the repetitive responses of a twin fiber preparation (fast and slow closers) is shown in Fig. 1. In *A* the action potentials of these two fibers elicited when they were stimulated simultaneously by an 100 msec. constant current are shown at relatively low amplifier gain. The stimulating voltage was about two times the rheobasic voltage for the slow closer. *B* represents the amplifier response to a 10 mv., 10 msec. signal at the gain used in *A*. Records *C*, *D*, and *E* show responses at high gain. Calibration of

the amplifier response at this high gain may be seen in *F*. *C* was obtained by stimulating at 6 times, *D* at 3.6 times, and *E* at 4.3 times the rheobasic voltage for the slow closer fiber. In records *C* and *E*, extreme cathodal depression

TABLE I

Rheobase Values of Single Lobster Motor Axons in Physiological Solution

Openers		Slow closers		Fast closers	
Fiber No.	Rheobase	Fiber No.	Rheobase	Fiber No.	Rheobase
	mv.		*mv.*		*mv.*
1	36	14	220	41	192
2	42	15	146	42	360
3	85	16	63	43	125
4	33	17	217	44	83
5	44	18	62	45	125
6	45	19	102	46	70
7	148	20	134	47	90
8	68	21	155	48	130
9	74	22	130	49	111
10	155	23	100	50	49
11	120	24	138	51	57
12	53	25	43	52	300
13	38	26	127	53	80
		27	26	54	160
		28	45	55	340
		29	150	56	48
		30	103	57	90
		31	65	58	250
		32	45	59	103
		33	70	60	75
		34	60	61	100
		35	50	62	70
		36	133	63	70
		37	55	64	215
		38	43	65	130
		39	58		
		40	175		
Means	72		101		137
Standard errors of mean	±12		±11		±18

may be seen with damped low amplitude potential oscillations. The repetitive response changed directly into the depressed state as the current was increased past a critical value.

Another aspect of constant current stimulation was the general loss of repetitive firing with fiber aging. This was apparently due to an increase of accomodation with time after preparation (Wright and Adelman, 1954).

Again, an order was seen in this phenomenon with respect to fiber type. Fast closers maintained their ability to respond repetitively to constant currents for shorter times after preparation than either slow closers or openers.

(*b*) *Rheobase Values.*—In determining the rheobase values of the individual motor axons, the oil-saline interface level (cathode) was set at approximately the same distance (0.5 cm.) from the anode on each fiber. The individual rheobase values so determined are given in Table I. The mean values were 72 mv. for the opener axons, 101 mv. for the slow closer axons, and 137 mv. for the fast closer axons. The appropriate standard errors of the means are given

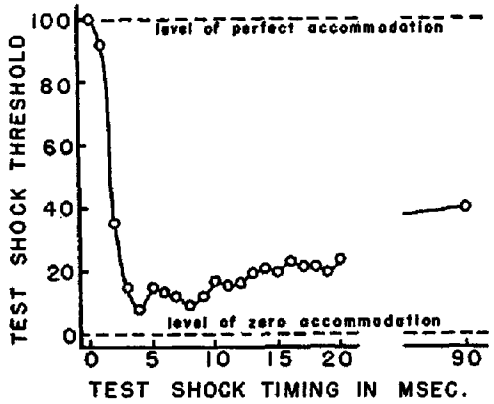

FIG. 2. Typical experimental curve for the variation in threshold as induced by subthreshold constant current conditioning. The "test shock" method. Ordinate, the test shock threshold in per cent of the unconditioned threshold. Abscissa; time interval between the "make" of the conditioning shock and the appearance of the test shock. Subthreshold conditioning was 90 per cent of the rheobasic voltage. See text.

in the table. There appears to be a small but significant difference among the rheobase values of the three fiber types. The order of these mean rheobase values was the same among the fiber types as the order of repetitive firing ability. The axonal type which fired the most repetitively also possessed the lowest rheobase values.

The considerable variation among the individual rheobase values of any one fiber type may have been due to varying stimulating electrode contact with the fibers from preparation to preparation, or to slight variations in the interelectrode distance. However, it would be strange, indeed, if there were no variance among fibers of a particular type.

(*c*) *Threshold Changes on the Application of Subthreshold Constant Currents.*—Measurements were made of the excitatory states as revealed by the variation in threshold to a very short duration "test shock" (Erlanger and Blair,

1931) when a cathodal subthreshold constant current was applied to these single fibers. A typical curve obtained from a slow closer fiber is seen in Fig. 2. Details of the method employed in obtaining and plotting such data are

TABLE II

Utilization Time Estimates (t_u) Determined from Curves Obtained by the "Test Shock" Method (See Text)

I. Twin fiber preparations (fast and slow closers)

Slow closers		Fast closers		Difference in t_u
R	t_u	R	t_u	
mv.	*msec.*	*mv.*	*msec.*	*per cent*
48	3.5	78	2.5	28.5
70	6.5	100	2.5	61.5
63	2.5	75	1.0	60.0
38	4.0	65	2.5	37.5
Means....55	4.1	80	2.1	46.9

II. All fiber types

Openers	Slow closers	Fast closers
t_u	t_u	t_u
msec.	*msec.*	*msec.*
4.5	6.5	3.5
10.0	2.5	2.5
9.0	6.5	2.5
24.0	4.5	4.5
10.0	6.5	1.0
	3.5	2.5
	6.5	1.0
	4.5	
	7.0	
	4.0	
	12.0	
	10.0	
Means.....11.5	6.2	2.5

R = rheobase, t_u = utilization time of the excitatory state.

given by Wright, Coleman, and Adelman (1955). In all fibers there was a tendency for the test shock threshold pattern to oscillate slightly with subthreshold conditioning. This mild oscillation may have been due to experimental error in determining the points. However, on repeated determinations the same oscillatory patterns could be seen again and again, and were always greater in magnitude than the expected errors inherent in the method. Also

this oscillation damped out with increasing time after the "make" of the constant current. Such oscillations in threshold, obtained by this method, have been seen by LeFevre (1950) in the squid giant axon, and have been recently noticed by Wright (1955) in lobster axons.

If the variations in threshold at the cathode seen on application of a subthreshold conditioning shock are related to the time course of the excitatory state as predicted by the two factor theories (Monnier, 1934) then the minimum threshold should represent the maximum of the excitatory disturbance, and thus occur at a time after the "make" of the conditioning shock equal to the utilization time, t_u. In determining t_u, the time corresponding to the minimum test shock threshold was taken as the utilization time. Table II

TABLE III

Time Constant of Accommodation (τ_2) Values for Single Lobster Motor Axons

Openers	Slow closers	Fast closers
75	100	14
89	85	90
100	90	80
85	83	14
106	98	77
85	60	83
Means.....90	86	60

shows the utilization times of the three types of fibers as determined by this method. A comparison between such values obtained from fast and slow closer axons prepared simultaneously is shown in part I of the table. About a 47 per cent difference between the utilization times of these fibers was found and is indicated in the table. In part II of Table II the individual t_u values of all fibers so estimated are shown. The mean values were 11.5 msec. for openers, 6.2 for slow closers, and 2.5 for fast closers. That these fibers are maintained at different levels of excitability is again evident from these data.

(*d*) *Excitability Constants.*—In Table III may be seen time constants of accommodation derived from data taken from the three types of fibers under identical conditions. Using exponentially rising currents (Solandt, 1936), estimates were obtained for Hill's λ or Monnier's τ_2 from the inverse slope of a plot of the threshold in rheobase units *vs.* the time constant of rise of the stimulating current. From the mean values obtained for τ_2 and the utilization time, t_u (obtained previously by the "test shock" method), the excitatory states were calculated for the different fibers from the general two factor excitability equations (Monnier, 1934). The relation between the utilization

time and the time constants of the excitatory state is:

$$t_u = \frac{\ln\left(\frac{\tau_2}{\tau_1}\right)}{\frac{1}{\tau_1} - \frac{1}{\tau_2}}$$

The calculated τ_1 values were 3.4 msec. for openers, 1.5 msec. for slow closers, and 0.5 msec. for fast closers. The observed τ_2 values were 90 msec. for open-

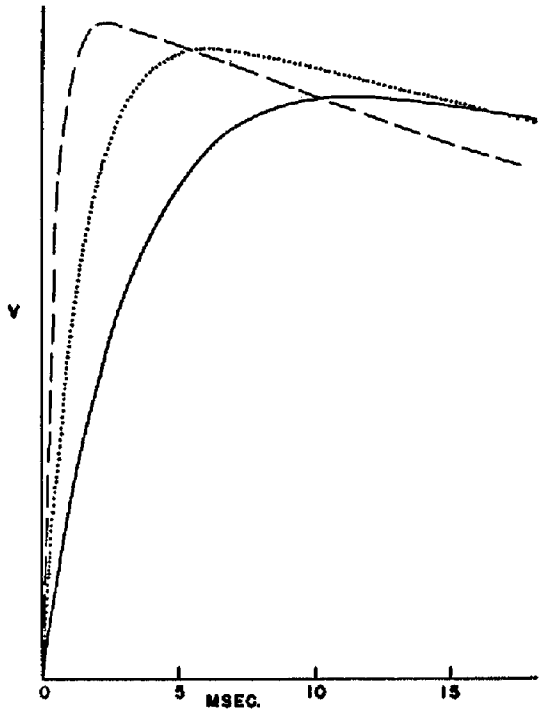

FIG. 3. Theoretical curves for the excitatory states of three types of motor axons. Constant current stimulation. See text. Solid line, opener; dotted line, slow closer; dashed line, fast closer.

ers, 86 msec. for slow closers, and 60 msec. for fast closers. LeFevre (1950) claimed that when $\tau_2/\tau_1 = 20$, there was about a 17 per cent overestimate of the real value of τ_2 when the estimates were obtained by the Solandt method. This overestimate follows directly from the assumption made by Hill (1936) in deriving the mathematical basis for the Solandt method, namely, that τ_2 must be many times greater than τ_1 in order to determine its value. Since almost all the τ_2/τ_1 ratios were relatively large, overestimates of τ_2 probably were small (somewhat less than 17 per cent).

It became apparent that it would be possible to plot the development of the excitatory state (V) based on the estimates for the time constants using the general equation for the excitatory state (Monnier, 1934):

$$V = Ri(e^{-t/\tau_2} - e^{-t/\tau_1}).$$

This equation predicts that for any applied constant voltage (Ri) the excitatory state (V) will rise to a maximum and then decline toward zero. The time course of the rise and fall of the excitatory state will be determined by the values of τ_1 and τ_2, which are time constants set by the tissue. By setting Ri equal to unity, $\tau_1 = 3.4$; $\tau_2 = 90$ for openers, $\tau_1 = 1.5$; $\tau_2 = 86$ for slow closers, and $\tau_1 = 0.5$; $\tau_2 = 60$ for fast closers, V was calculated for various arbitrary values of t (msec.). In Fig. 3 the V values so obtained are plotted

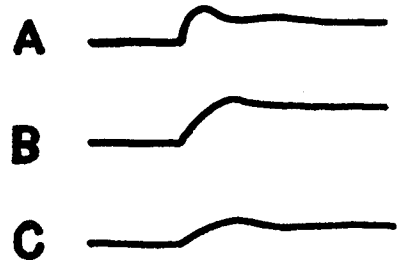

FIG. 4. Typical "local" or "initial" potentials obtained with just subthreshold constant current stimulation. Tracings of original records. See text. *A*, fast closer. *B*, slow closer. *C*, opener.

against time and represent the theoretical excitatory states for the three fiber types.

(*e*) *Local Responses.*—In a few cases, by lowering the oil-saline interface to the ground electrode, responses could be recorded directly from the stimulating cathode. Opener fibers normally showed a slowly developing local response, with its peak or maximum developing from 5 to 15 msec. from the beginning of an applied subthreshold constant current. There was a general similarity between the time course of the local response and the development of the excitatory state. With subthreshold constant current stimultation, the local response occasionally showed mild damped oscillations following the initial development. Fig. 4 is representative of such local potential recordings. *A* represents the local potential of a typical fast closer fiber; *B* represents the local potential of a typical slow closer fiber, and *C* represents the local response of a typical opener fiber. The time intervals between the "make" of the current and the peak of the local potential were 2.5 msec. for the fast closer, 5 msec. for the slow closer, and 6 msec. for the opener. These values are similar to the values for the excitatory state utilization times for the three

fiber types as determined by the "test shock" method. Consequently, there seemed to be a rather close parallelism between the development of the excitatory state and the development of the local potential.

DISCUSSION

From the foregoing results it seems apparent that the three anatomically different types of lobster motor nerve fibers possess different excitabilities. It is suggested that the more lengthy repetitive discharges seen in openers are a result of longer lasting and less accommodative local excitatory states. Prosser and Chambers (1938) found that the chronaxies of uncut giant fibers of the stellar nerves of the squid, stimulated *in situ*, were less than the chronaxies of uncut fin nerves of the same animal. Thus Prosser and Chambers were able to demonstrate a difference in excitability for anatomically different invertebrate nerve fibers.

Hodgkin (1948) found that *Carcinus* axons could be classified into three groups with respect to their ability to fire repetitive responses to constant current stimuli. Hodgkin found that the group of fibers which showed the widest range of frequency of firing, upon D.C. stimulation, also showed the longest utilization times, indicating either an absence of accommodation or an extremely slow primary excitable process. By the same token, the axons with the highest rheobases and the lowest safety factors, were shown to be either non-repetitive or only mildly repetitive upon constant current stimulation. Hodgkin also implied that there was a marked resemblance between the potential changes which precede the first spike in a repetitive sequence and those which precede any other spike in that sequence. Consequently, Hodgkin concluded that the frequency of repetitive firing with mild cathodal constant stimuli was determined by the response or utilization time rather than by the refractory period in the class of axons firing at slow frequencies (class I). However, in class II fibers a pronounced supernormal phase was observed. These axons tended to show little variation in frequency of repetitive firing upon variation in stimulus strength (in contradistinction to class I axons). Hodgkin concluded that the frequency of firing was determined by the form of the recovery curve and the rate of growth of the local response (interaction between supernormality and utilization time). However, he cautioned that it would be impossible to predict the frequency of the repetitive discharge from the recovery curve alone.

It would seem that the three classes of axons described by Hodgkin were not truly anatomical classes discovered by physiological means. Since no attempt was made by Hodgkin to present an anatomical-physiological relationship for his axons, close comparison between the fiber types used in this work and those used by Hodgkin becomes difficult. For the most part, opener fibers and slow closer fibers were similar to class I *Carcinus* axons, while fast

closer fibers more closely resembled class III *Carcinus* fibers. Since the supernormal phase was not estimated in this work, and since none of the lobster fibers showed such narrow limits of repetitive frequencies as class II *Carcinus* axons, comparisons of the lobster fibers with these fibers could not be made.

Easton (1952) showed in bundles of nerve fibers prepared from the leg nerve of *Cancer magister*, that different strength-duration curves corresponded to different sized action potentials produced by fibers in these bundles. The larger recorded spikes had shorter latencies, higher rheobases, and shorter chronaxies than the smaller recorded spikes. Whether Easton was dealing with fast and slow closer fibers or some other unknown fibers remains obscure, particularly since Easton made no attempt to discover which fibers in his prepared bundles were responsible for the different spikes. However, Easton guessed that the larger spikes, rather than the smaller spikes, belonged to larger diameter fibers.

Wright and Coleman (1954) and Wright and Adelman (1954) demonstrated similar differences in excitability among fast closers, slow closers, and openers in lobsters and crayfish as has been shown in this report. Wright, Coleman, and Adelman (1955) found that when these same fiber types were stimulated in sea water with a distance of 1 mm. between the oil-saline interface (cathode) and the anode, the rheobase was on the order of 20 mv. Since the resistance between cathode and anode was directly proportional to the interelectrode distance, increasing this distance to 5 mm., as was done in this work, should result in a fivefold increase in the rheobasic voltage, assuming that the rheobasic amperage is a constant for a fiber type. It follows then, that in terms of the effective stimulating current applied to the tissue, the rheobase values obtained in this work (Table I) are of the same order of magnitude as those obtained by Wright, Coleman, and Adelman (1955).

Since it has been indicated that the threshold of these fibers is on the order of 100 mv. for an interelectrode distance of 5 mm., there appears to be no conflict between the rheobase values in Table I and the "safety factor" concept. Since it takes a rheobasic voltage comparable to the maximum voltage developed by the spike (100 mv.) to stimulate a spot on the nerve 5 mm. away from the current source, it seems very likely that a shorter duration current (the spike is about 1 msec. in duration) of the same voltage would be able to stimulate a region of the nerve membrane some millimeters in advance of the active current source. In terms of current flow, the region in advance of the impulse would be the source and the impulse would be the sink.

That there is some underlying rhythmical process in nerve seems quite probable in view of results presented by Adelman (1956 *a*). In a sense, the local potential may act much like a "pacemaker," in which its frequency, amplitude, and damping regulate the production of the action potential. Recently, Wright and Adelman (1956) showed that when a normally repetitively firing axon becomes non-repetitive the local response is shortened to

appear similar to the normal local response of a normally non-repetitively firing fiber. Wright and Coleman (1954) have revived the concept developed earlier by Arvanitaki (1943) and Brink, Bronk, and Larrabee (1946) that the local impulses are probably the "pacemakers" for the repetitive spike discharge. Small potential oscillations were recorded across the membrane of the squid giant axon by Marmont (quoted by Cole, 1949), and by Hodgkin and Huxley (1952). Usually associated with the action potential and preceding it to some extent was a small potential change referred to by Hodgkin (1938) as the "local potential" and by Wright and Coleman (1954) as the "initial potential." An applied current can be thought of as a condition giving rise to a local potential (Hodgkin and Rushton, 1946) which then triggers the spike or action potential. In the case in which low amplitude potential oscillations resulted from cathodal depression these may have been brought about by the applied current depolarizing the membrane to such an extent that each successive repolarization against the continually applied current was less successful in developing the local potential to its trigger value. This can be considered analogous to an overly increased sodium inactivation (Hodgkin and Huxley, 1952). The possibility that increased current strengths increased the effective cathodal area was always present. However, Adelman (1956 *b*) has shown that as an applied cathode became more diffuse with respect to an applied anode, single muscle fibers became more repetitive to constant current stimulation.

The characterization of the excitabilities of the three types of fibers by the use of real time constants is unwarranted perhaps. On manipulation of the two factor equations to produce a critically damped state, it is found that τ_1 must equal τ_2. However, to produce oscillations the time constants must be conjugately complex. Thus by including real values for τ_1 and τ_2 in the two factor equations the slightly underdamped excitable process is approximated with an overdamped curve. The values of these constants then are only a substitute for the "true constants" which remain complex (*cf.* Cole, Antosiewicz, and Rabinowitz, 1955 p. 500). Certainly, both the excitatory states and the local potentials determined in this work were mildly oscillatory, and therefore the estimated real constants are merely crude approximations.

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SUMMARY

1. Three anatomically different types of lobster motor axons have been shown to possess different excitabilities under identical conditions.

2. The type, openers, which fired the longest trains of repetitive responses

also had the lowest rheobase values, the longest utilization times, and the least accommodation.

3. A relationship between the time course of the local potential and the time course of the excitatory state has been disclosed.

4. The characterization of the excitatory state by simple two factor equations has been discussed and found to be only approximate since both the excitatory state and the local response tend to be somewhat oscillatory.

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