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STRUCTURE AND ELECTRICAL PROPERTIES OF EYE MUSCLES IN CAVE- AND SURFACE-DWELLING CRAYFISHES

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SUMMARY

The morphologies and passive electrical parameters of fibres in two eye muscles of a surface- and a cave-dwelling crayfish were compared. In the cave-dwelling form the muscles contained fewer fibres, of less diameter, and hence had a smaller cross-sectional area. Current-voltage relationships were similar in both species. Input resistance was higher in the cave-dweller, but the difference was not as great as would be expected on the basis of geometry alone. Accordingly, the specific membrane resistance of muscle fibres in the cave-dweller is 50–60% smaller than that in the surface-dweller. This may account partially for the observation that identified excitatory junctional potentials in muscles of cave- and surface-dwellers have similar amplitudes. We conclude that a functional oculomotor system is maintained in cave-dwelling crayfish, and that this system confers some positive selective advantage.

INTRODUCTION

Organisms that invade and become permanent residents of the cave habitat evolve, after many generations, a constellation of biochemical, physiological, and morphological adaptations. Most such animals lack cuticular pigmentation, possess bodies which are generally less robust than the putative stem line, are extremely sensitive to vibrational disturbance, and are usually blind. The presence of such commonly held adaptations among not only congenerics but also entirely unrelated groups of animals, such as fish, salamanders, and crayfish, attests to the powerful influence of the selective pressures which are peculiar to the cave environment.

Among the most pervasive of structural adaptations in cave-dwelling animal species is the reduction of visual structures, the eyes of most permanent cave-dwellers (troglobites) being either very small or altogether absent. Almost nothing is known about the cellular changes or mechanisms which underlie visual reduction in these animals, although the prolonged absence of light is believed to have a direct deleterious effect upon the primary visual receptor cells of some surface-dwelling forms (Eguchi & Waterman, 1966; Roach & Wiersma, 1974). We reasoned that the neurones and

muscles of the troglobite oculomotor system, whose structural and functional attributes cannot be directly influenced by the absence of light, might provide an interesting study in evolutionary change. The primary function of these structures in surface-dwelling (epigean) animals is the stabilization of retinal images; their functional importance in blind cave-dwelling species must therefore be drastically curtailed. To pursue this line of reasoning, selective pressures must eventually lead to the disruption of the oculomotor apparatus, if for no other reason than that anomalies arising from random mutational changes may no longer be selectively eliminated from the genome by acute environmental influences. Examination of the oculomotor system in cave-dwelling species may consequently reveal those sorts of structural and functional cellular changes which occur with highest frequency, perhaps thereby indicating the most probable channels through which neural evolution can occur. To this end studies were started of the oculomotor arrangement in both surface-dwelling and cave-dwelling species of the crayfish, *Procambarus* (Mellon, Tufty & Lorton, 1976; Mellon, 1977*a*, *b*; Mellon & Lorton, 1977). In the course of these studies (Mellon, 1977*b*) we found that neither the eye muscles nor their motor neurones in a cave species (*P. erythrops*) exhibited any gross abnormalities. However, all of the eye motor structures in this species are very much reduced in size. In this article we compare the electrical and anatomical properties of two types of eye muscle of surface- and cave-dwelling crayfish.

MATERIALS AND METHODS

Experiments and observations were done on adult epigean *Procambarus clarkii* obtained from a commercial supply house, and on adult troglobitic *P. erythrops*, collected in the field in Suwannee County, Florida.* Animals were kept in fresh water: *P. clarkii* in communal tanks, and *P. erythrops* in individual containers. Anatomical observations were made on muscle material fixed *in situ* in 2% glutaraldehyde, washed in buffered sucrose, post-fixed in 1% osmium tetroxide, and embedded in Epon. Thick (1 μM) sections were cut with glass knives on a Porter–Blum microtome and stained with methylene blue. Area measurements of muscle fibres were made from photographs of sectioned material, using a computerized planimeter.

Physiological experiments were performed on dissected preparations at room temperature. Animals were decapitated, and the head was pinned ventral side down to the floor of a dissection chamber flooded with Van Harreveld's solution. Small pins were used to secure an eye in an extended position, and subsequently, either muscle 15 or 16 was exposed by dissection and removal of overlying tissues.

Electrophysiological measurements of muscle fibre input resistance (R_0) and fibre length constant (λ) were made directly using pairs of glass micropipette electrodes filled with 2·5 M-KCl. Controlled constant current pulses of 600–700 ms duration were passed through one electrode while the membrane voltage change was measured

* Observations on the properties of *Procambarus erythrops* eye muscles had to be terminated when it was recognized that continued collecting of these narrowly distributed animals seriously threatened the survival of the population. I am indebted to Dr Richard Franz of the Florida State Museum at Gainesville for advising me of the probable consequences of continued exploitation of this source of cave-dwelling crayfish.

with the second. To obtain individual curves of $E:I$, both electrodes were inserted into the same muscle fibre as close together as possible (usually about 50 μm separation) and calibrated steps of inward current were alternated with outward steps. Currents larger than 100 nA were not used. Measurements of membrane length constant, λ, were obtained by recording the membrane voltage response at several different sites on a fibre to a known, 600 ms pulse of inward current. In these instances, initial electrode separation was usually 75 μm, and R_o was obtained by extrapolation of the voltage versus distance curve (e.g. Fig. 5) back to 0 mm.

Supplies of cave crayfish became unavailable before direct length constant measurements were obtained from muscle 16. Consequently the values for specific transmembrane resistance (R_m) of muscle 16 in *P. erythrops* were calculated assuming specific internal resistance (R_i) values obtained from homologous fibres in *P. clarkii*. While the calculated values for this parameter in cave animals are thus less certain than in the case of *P. clarkii*, it should be pointed out that the values for R_i measured from muscle 15 in *P. clarkii* (174 Ω cm) and *P. erythrops* (166 Ω cm) are very similar.

Excitatory junction potential (EJPs) in muscle 15 were evoked by direct electrical stimulation of the optic nerve motor bundle. Muscle 15 is supplied by two of the three giant motor axons which mediate rapid eye withdrawal in these animals (Mellon, 1977*a*). The largest of these, G_1, was found previously to have the lowest threshold to electrical stimulation, and this criterion was used in the present study to establish a standard postsynaptic response in muscle 15 fibres of *P. clarkii* and *P. erythrops*.

RESULTS

The eye structures of *P. erythrops* are very much smaller than those of *P. clarkii*, the corneal region being confined to a circumscribed area at the tip of the eyecup, while that in *P. clarkii* occupies the terminal two-fifths of the eyecup (Fig. 1). It is obvious from a cursory examination of Fig. 1 that all the 11 eye muscles in the cave animals must be much reduced in size compared to those of the surface-dwelling forms. As shown in Fig. 2, this difference results from the presence of fewer fibres, of smaller average diameter, in *P. erythrops* than in *P. clarkii*. Fig. 3 displays calculated radii for circumferential muscle fibres (obtained from area measurements by assuming each fibre to be a perfect cylinder). Geometrical measurements were confined to circumferential fibres because electrical observations had previously been made only on these fibres, and also because, by visual inspection alone (Fig. 2), there are general differences in size and shape between the fibres at the periphery and those at the muscle core.

A bimodal distribution of fibre radii is exhibited in both muscles, the large diameter group in each case having a peak at about twice the radius of the smaller. Since no obvious similar distributions were evident among our input resistance measurements, the data may represent random diameter variations among an otherwise fairly uniform population of fibres. We did not, however, examine this possibility.

Series of current–voltage (E–I) curves were obtained for muscle 15 in both crayfish species. Fig. 4 shows the average data for each species. The slope resistance for the muscle fibres in *P. erythrops* was consistently larger than for *P. clarkii*, though not as large as the smaller fibre size would allow one to predict (heavy solid curve). This point is discussed further below.

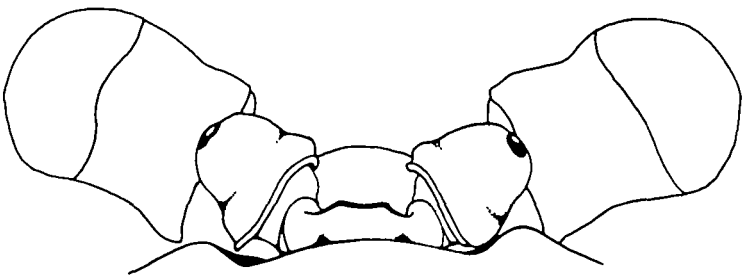

Fig. 1. Dorsal view of the eye structure of *P. erythrops* superimposed upon an outline drawing of the comparable organs in *P. clarkii*. The lengths of the cephalothorax of the two animals were identical (3·5 cm).

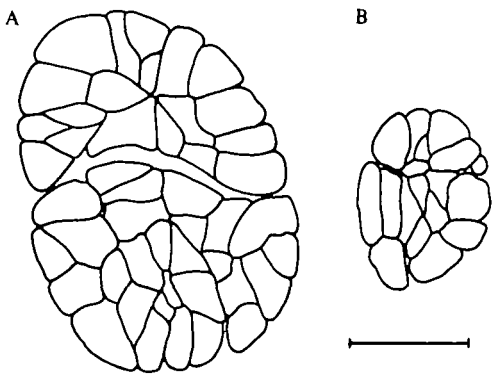

Fig. 2. Transverse, one micrometer, section of muscle 15 in (A) *P. clarkii* and (B) *P. erythrops* taken from approximately the same regions of the respective muscles. The margins of the individual fibres have been outlined in ink for greater clarity. The muscles appear to be split due to the fact that each has a dual origin, as described elsewhere (Mellon, 1977*a*). Calibration bar: 250 μm.

Direct measurements of the fibre length constant, λ, were made in six fibres of muscle 15 of each crayfish species (Table 1). The specific resistance, R_i, of the muscle fibre interior and the specific transmembrane resistance, R_m, were then calculated using the following relationships:

$$R_i = \frac{2\pi}{\lambda}\left(\frac{d}{2}\right)^2 R_o, \tag{1}$$

$$R_o = \frac{1}{\pi}\left[\frac{R_m \cdot \mathrm{Ri}}{d^3}\right]^{\frac{1}{4}}, \tag{2}$$

where d is the mean value of fibre diameter.

The smaller diameter in the muscle 15 fibres of *P. erythrops* compared to *P. clarkii*, the resultant higher input resistance, and the calculated reduction in R_m, lead to a limited length constant in the muscles of the cave animals. This is shown in Table 1 and also in Fig. 5, the examples graphically illustrating the difference in the capacity for electrotonic spread of transmembrane voltage in the two species.

In order to ascertain whether or not the increased input resistance of the muscle

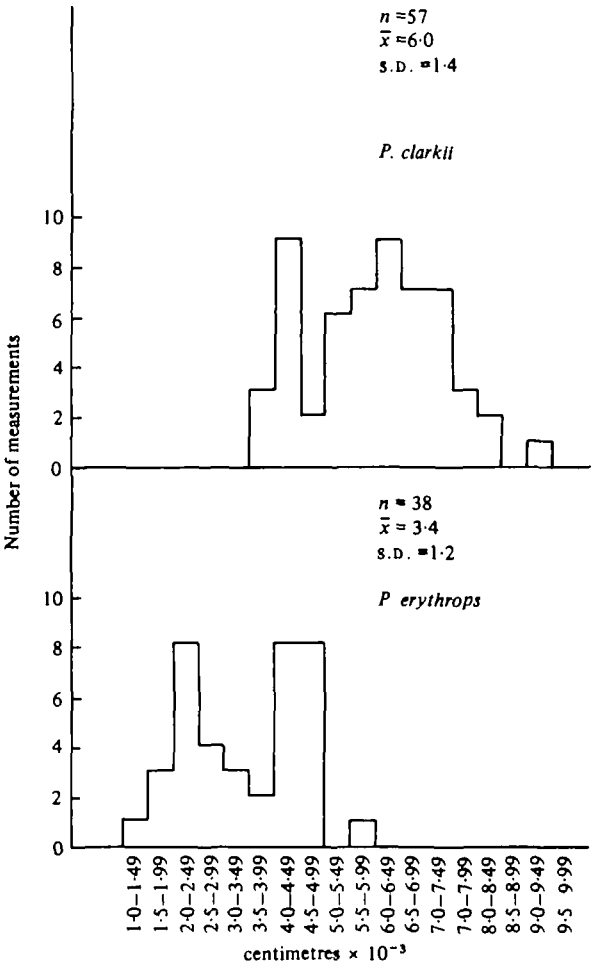

Fig. 3. Histograms showing the distribution of sizes (as radii) of peripheral fibres in muscle 15 of *P. clarkii* and *P. erythrops*.

fibres in *P. erythrops* could be accounted for exclusively on the basis of their smaller diameters, we constructed a curve of *E v. I* from R_0 values obtained by substituting the mean diameter of cave-dwelling muscle 15 fibres in equation (1). The resulting solid curve of Fig. 4 suggests that parameters of the muscle other than size may be different in the cave-dwelling form, and, indeed, the data for *P. erythrops* in Table 1 indicate that the specific membrane resistance of muscle 15 fibres is a little more than half as large as in *P. clarkii*.

Very similar findings were obtained in muscle 16 of the two crayfish species. The muscle cross section in Fig. 6 again shows a substantial size difference, based upon total number of muscle fibres as well as on individual fibre size. Electrical measurements with fibres in muscle 16 are graphically illustrated in Fig. 7. As found previously the peculiar characteristics of the *E–I* relation are preserved in the cave crayfish, showing in this case a pronounced hyperpolarizing activation such as is seen in other crustacean muscles (Atwood, 1963; Grundfest, 1966). Once again the individual

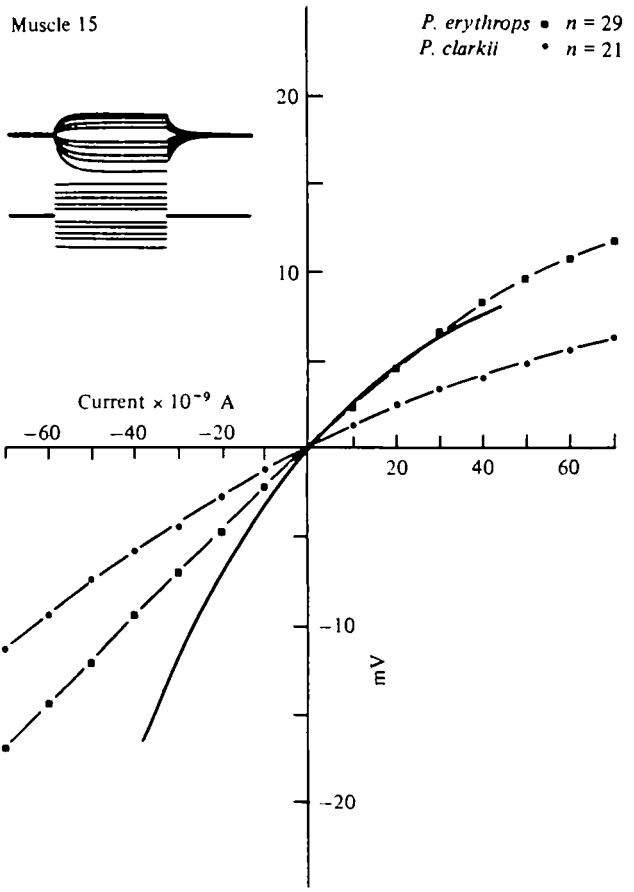

Fig. 4. Curves of the relationship between injected current and membrane voltage change in fibres of muscle 15 of both the surface dwelling (●) and the cave-dwelling (■) species. The heavy solid curve was constructed from calculated R_m values at each voltage point on the curve for *P. clarkii*, but using the mean diameter of *P. erythrops* fibre in calculating the several values of R_0 at these points.

fibres of the cave-dwelling muscle possess a higher input resistance than those of the epigean animals (Table 2). Here too, however, the extent of the difference is less than would be expected from size difference alone. Using values of $3{\cdot}82 \times 10^{-3}$ and $1{\cdot}93 \times 10^{-3}$ cm for the epigean and troglobitic muscle diameters, respectively, a theoretical relationship based upon the smaller diameter cave muscle fibre was constructed and is shown on the heavy solid curve in Fig. 7. As with muscle 15 the difference in the two curves suggest that some other parameter as well as size may be different in the two muscles.

As direct measurement of fibre length constants could not be obtained from muscle 16 in *P. erythrops*, R_m was calculated from the input resistance measurements and using a value of 150 Ω-cm for R_i in the solution of equation (2). Justification for the use of this value for R_i is discussed in the Methods section above. A further possible difficulty devolves from the overall length of the muscle fibres in relation to their length constants. When this ratio becomes small, a fibre no longer can be considered

Table 1. *Muscle* 15

Cell	R_o (Ω)	λ (cm)
	Procambarus clarkii	
6. vi. 77. 1	$9{\cdot}05 \times 10^4$	0·200
6. vi. 77. 2	$1{\cdot}00 \times 10^5$	0·220
6. vi. 77. 4	$8{\cdot}00 \times 10^4$	0·220
8. vi. 77. 2	$1{\cdot}25 \times 10^5$	0·090
8. vi. 77. 3	$1{\cdot}20 \times 10^5$	0·127
8. vi. 77. 4	$2{\cdot}30 \times 10^5$	0·120
Mean	$1{\cdot}24 \times 10^5$	0·163
S.D.	$\pm 0{\cdot}54 \times 10^5$	±0·057
R_i	174 Ω-cm	
R_m	1532 Ω-cm^2	
	Procambarus erythrops	
21. vi. 77. 1	$1{\cdot}3 \times 10^5$	0·105
21. vi. 77. 2	$2{\cdot}6 \times 10^5$	0·119
23. vi. 77. 1	$1{\cdot}9 \times 10^5$	0·081
25. vi. 77. 1	$2{\cdot}6 \times 10^5$	0·078
25. vi. 77. 2	$2{\cdot}0 \times 10^5$	0·078
25. vi. 77. 3	$2{\cdot}0 \times 10^5$	0·081
Mean	$2{\cdot}07 \times 10^5$	0·090
S.D.	$\pm 0{\cdot}49 \times 10^5$	±0·017
R_i	166 Ω-cm	
R_m	796 Ω-cm^2	

Table 2. *Muscle* 16

Cell	R_o (Ω)	λ (cm)
	Procambarus clarkii	
8. vi. 77. 1	$2{\cdot}7 \times 10^5$	0·150
8. vi. 77. 2	$2{\cdot}8 \times 10^5$	0·150
8. vi. 77. 3	$2{\cdot}8 \times 10^5$	0·145
13. vi. 77. 2	$1{\cdot}5 \times 10^5$	0·195
13. vi. 77. 4	$4{\cdot}0 \times 10^5$	0·160
22. vi. 77. 1	$2{\cdot}3 \times 10^5$	0·192
Mean	$2{\cdot}68 \times 10^5$	0·165
S.D.	$\pm 0{\cdot}81 \times 10^5$	±0·022
R_i	150 Ω-cm	
R_m	2139 Ω-cm^2	
	Procambarus erythrops	
6. vi. 77. 1	9×10^5	
20. vi. 77. 3	$3{\cdot}5 \times 10^5$	
20. vi. 77. 4	4×10^5	
28. vi. 77. 2	3×10^5	
28. vi. 77. 3	2×10^5	
20. vi. 77. 1	3×10^5	
20. vi. 77. 2	7×10^5	
Mean	$4{\cdot}5 \times 10^5$	
S.D.	$\pm 2{\cdot}5 \times 10^5$	
R_i	150 Ω-cm	
R_m	766 Ω-cm^2	

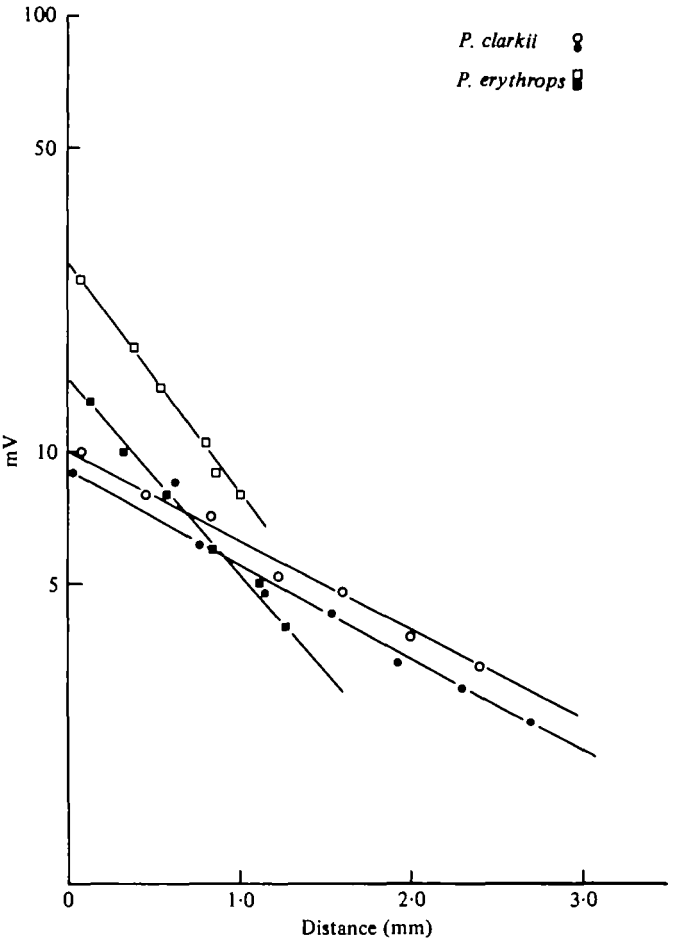

Fig. 5. Examples of electrotonic voltage spread in individual fibres of muscle 15 in *P. clarkii* and *P. erythrops*. The curves are essentially exponential.

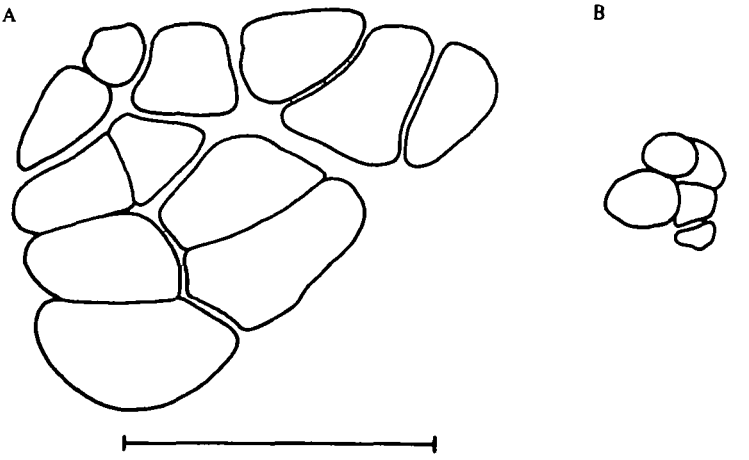

Fig. 6. Transverse sections of muscle 16 in *P. clarkii* (A) and *P. erythrops* (B). Calibration bar is 250 μM.

Table 3

E_R (mV)	R_o (Ω)	E_R (mV)	R_o (Ω)
64	2×10^5	63	8×10^5
54	4×10^5	68	6×10^5
45	2×10^5	76	$5{\cdot}5 \times 10^5$
69	6×10^5	66	4×10^5
60	3×10^5	58	3×10^5
78	$5{\cdot}5 \times 10^5$	63	$3{\cdot}5 \times 10^5$
67	$7{\cdot}5 \times 10^5$	68	3×10^5
70	6×10^5	73	$3{\cdot}5 \times 10^5$
64	5×10^5	65	3×10^5
71	6×10^5	69	3×10^5
76	5×10^5		

E_R (mV)	R_o (Ω)
Mean = 66	$4{\cdot}5 \times 10^5$
S.D. = ±7·7	$\pm 1{\cdot}7 \times 10^5$

n = 0·43

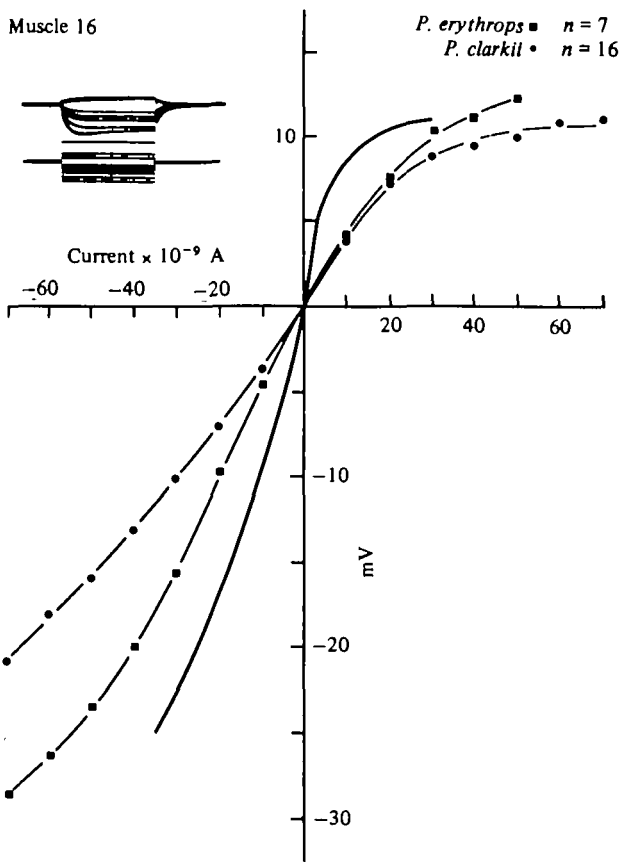

Fig. 7. Current–voltage curves for muscle 16 fibres in both crayfish species. The heavy solid line curve is again a theoretical construct generated by using the mean fibre radii from *P. erythrops* and the point values of R_m calculated for *P. clarkii*.

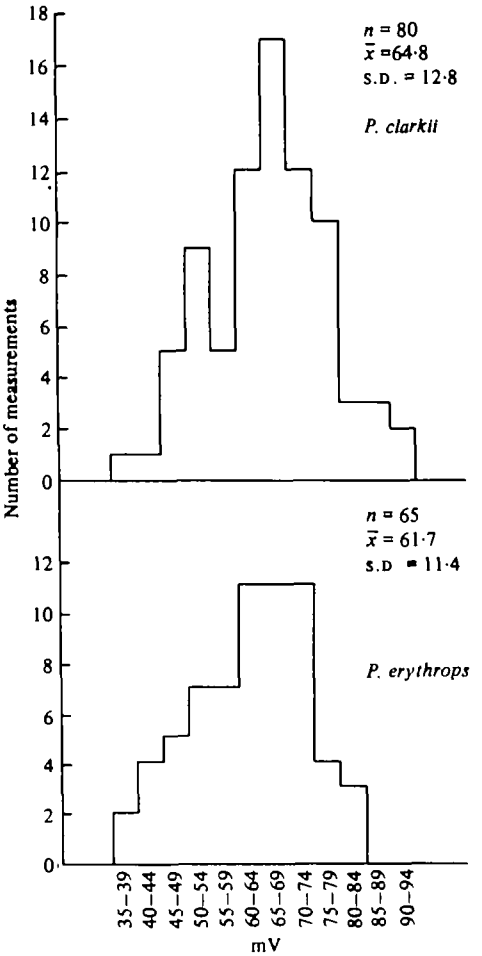

Fig. 8. Comparison of resting membrane potential distributions in fibres of muscle 15 from *P. clarkii* and *P. erythrops*.

an infinite cable, and modification of equations (1) and (2) are required (e.g. Fatt & Ginsborg, 1958). However, inspection of the voltage versus distance curves for both muscle 15 in *P. clarkii* and *P. erythrops*, and muscle 16 in *P. clarkii*, showed this relationship to be strictly exponential. We therefore assume that the use of the above equations in unmodified form is warranted.

Table 2 illustrates some mean values for the various parameters of muscle 16 fibres. R_m of the fibres in the troglobitic species exhibits even greater difference from its epigean counterpart in this muscle than in muscle 15. One may therefore be able to predict that a generalized reduction in transverse membrane resistance has accompanied size reduction in the fibres of all eye muscles in this species.

Resting potential in muscle 15 was found to be different between the two species, with a lower mean value for *P. erythrops* than *P. clarkii* (Fig. 8). To test whether this was due to the current and/or voltage electrodes causing greater damage to the relatively small fibres of *P. erythrops*, we compared individual resting potential and

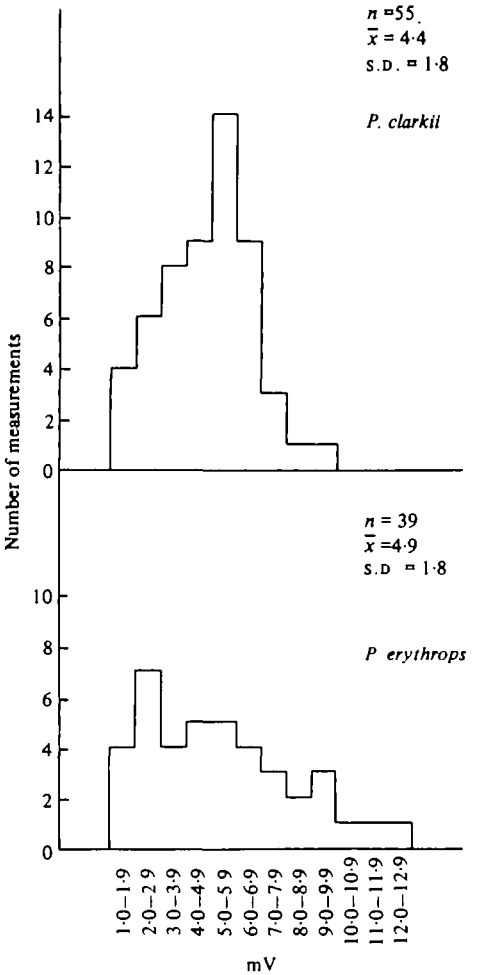

Fig. 9. Amplitude distribution of early threshold EJPs in muscle 15 from the surface-dwelling and the cave-dwelling species.

input resistance measurements obtained from 21 muscle 15 fibres from *P. erythrops* (Table 3). A correlation coefficient of 0·43 was obtained, suggesting that there is less than a 20% chance in any fibre that a low reading of resting potential will be accompanied by an equally low value of input resistance. We concluded that, whatever the cause of the reduced resting membrane potentials in *P. erythrops* muscle fibres, it is probably not due to physical damage induced by the measuring electrodes.

Because the overall input resistance of the fibre in muscle 15 of *P. erythrops* is larger than that of its epigean counterpart, one might expect that the voltage drop occasioned by synaptic currents would also be larger. However, the distributions of threshold excitatory junction potentials (EJPs) in the two species are essentially very similar, as shown in Fig. 9. This suggests that the synaptic current generated by axon G_1 is less in *P. erythrops* than it is in *P. clarkii*.

DISCUSSION

The current–voltage relationships revealed in the present study of crayfish eye muscle are similar to those obtained with other crustacean muscles. Muscle 15 generates fast twitch contractions. It exhibits large EJPs in response to motor axon stimulation, and its fibres have the largest diameter of all the eye muscles. This muscle falls into the 'A-type' category of Atwood (1963), and the *E–I* curve exhibits a gradually increasing slope (increasing resistance) in the hyperpolarizing range of membrane potential, as well as the conventional 'delayed' rectification to increasing outward currents.

Muscle 16 exhibits initially small, but markedly facilitating, EJPs. The fibres are apparently not electrically excitable. This muscle is most easily placed under Atwood's category B. Delayed rectification increases sharply above a normal range of depolarization, but the response of the membrane to hyperpolarizing currents is the opposite of that exhibited by muscle 15. In muscle 16 increased membrane potential brings about a decrease in slope and, consequently, a reduced transmembrane resistance. This phenomenon has been termed hyperpolarizing activation by Grundfest (1966) and probably represents a voltage-dependent increase in membrane chloride conductance.

It is remarkable that the characteristics of the current–voltage relationships in these two rather disparate muscle types have been conserved in the reduced eyes of the cave-dwelling crayfish examined in this study. While the muscles have both undergone severe reduction, both in fibre number and in fibre size, and the changes in specific transmembrane resistance notwithstanding, the functional properties of their membranes are hardly changed.

Eye structure in *Procambarus erythrops* has undergone relatively rapid evolutionary modification. In response to the selective pressures peculiar to the subterranean environment, nearly the entire retina and much of the four optic ganglia have degenerated (Mellon, 1977*b*). What remains of these structures is housed in an eyecup of unusually small size. Similarly, the size of the oculomotor apparatus has been reduced. Muscle fibre number has been reduced by more than sixty percent; fibre diameter has been decreased by one-third to one-half, and motor axon diameter has been halved. Despite these modifications, the functional integrity of the troglobitic oculomotor system has been retained. The various eye reflexes are completely intact (Mellon, 1977*b*) and, as shown in the present work, there is an excellent functional match between the motor neurones and the individual muscles that they supply. To be more precise, synaptic potentials in muscle 15 of *P. erythrops* have an amplitude distribution which is nearly identical with that in the homologous muscle of *P. clarkii*, despite the smaller diameter in the cave-dwelling form which may be expected to lead to a higher input resistance. It may be argued that this match is merely due to a fortuitious, coupled size reduction in muscle and motor nerves, as the smaller size of the axon terminals would be expected to result in a smaller output of neuromuscular transmitter (Kuno, Turkanis & Weakly, 1971). While this may be so, the curves in the graphs of Figs. 4 and 7 suggest that the expected, geometry-dependent, increase in input resistance has not occurred. Apparently this is due to a 50–60% smaller specific membrane resistance in the muscle fibre of the cave animals. It therefore seems that

the oculomotor neurones and muscles of *P. erythrops* have undergone physiological as well as geometrical adaptation to suit the reduced size of the eyes in this species.

The value of the oculomotor system in *P. erythrops* may be in spatial orientation. Without visual cues, it is clear that all means of establishing bodily orientation in space become especially important to cave-dwelling animals. The precision of eyestalk reflexes – both static and dynamic – and the range of different sensory modalities that influence these reflexes means that eye position could be used as a reliable indicator of body movement, body position with respect to the gravitational field, and/or leg position with respect to the body. However, no independent sensory systems capable of accurately measuring eye-body angle or eye movement in crustaceans are known and thus, as with many other features of these intriguing animals, any possible role for eye movement in spatial orientation control remains speculative.

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