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CHROMATOPHORE MOTOR UNITS IN *ELEDONE CIRRHOSA* (CEPHALOPODA: OCTOPODA)

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SUMMARY

Innervation of chromatophore muscles of the octopus *Eledone cirrhosa* was investigated by stimulating nerve bundles in the skin with a suction electrode and monitoring chromatophore movements with a photo-cell or a video camera. Attention was focused on the organization of the chromatophore muscle fibres into motor units. Individual muscle fibres respond to single electrical impulses with twitch-like contractions that do not facilitate with repetition, but summate to a smooth tetanus at about 10–15 Hz. At tetanic frequency, the degree of expansion of single chromatophores is always maximal. However, the number of expanded chromatophores can be graded by variations of either the stimulus voltage or frequency. Individual chromatophores and probably individual muscle fibres are part of several motor units. Chromatophores forming a given motor unit are found among chromatophores served by other motor axons. The motor units apparently form precise parts of natural patterning.

INTRODUCTION

The chromatic patterns of cephalopods are unique within the animal kingdom: they rely on chromatophores which are true organs made of a central pigment-containing cell (pigment cell) surrounded by ten to twenty radially arranged muscle fibres (Hofmann, 1907*a*; Cloney & Florey, 1968; Froesch, 1973; Brocco, 1977). Contraction of radial muscle fibres produces expansion of the chromatophore while their relaxation leads to its retraction. The activity of the chromatophore muscle fibres is under the direct control of motor axons running from the chromatophore lobes in the brain (Boycott, 1953, 1961; Young, 1971). This system allows cephalopods to change chromatic patterns with a speed, variety and subtlety impossible in hormonally controlled chromatophore systems (Packard & Sanders, 1971; Packard & Hochberg, 1977).

Since the chromatic patterns reflect the activity of a true neuromuscular system, they can be used to study neuromuscular relationships. The particular radial arrangement of the muscle fibres around the pigment cell allows single fibres to be precisely located and their activity monitored individually. Furthermore, the mechanical

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behaviour of several muscle fibres can be recorded simultaneously and individually by simple optical devices monitoring the effect of the muscle contraction on the expansion of the pigment cell. Thus, the chromatophore system is suited for studies at intermediate levels of organization (e.g. groups of muscles or motor units), an approach that is more difficult in muscles where individual fibre activity can be detected only with intracellular electrodes.

Chromatophore muscles show the following characteristics, shared with other molluscan muscles (see Hoyle, 1983): mechanical activity mediated by graded postsynaptic potentials, multiple innervation of individual fibres, electrical coupling between at least some of the muscle fibres, spontaneous activity, phasic and tonic contractions (Hofmann, 1907*b*, 1910; Bozler, 1928, 1931; Florey, 1966, 1969; Florey & Kriebel, 1969).

Attempts have been made to relate the chromatic patterns to the underlying organization of the nervous system. In cuttlefish mantle, stimulation of nerve branches has shown that chromatophores innervated by a single axon are randomly scattered among chromatophores innervated by other axons (innervation type B, Maynard, 1967) whereas, in the dorsal skin, motor units consist of the chromatophores involved in the same pattern or part of a pattern. Stimulation of the skin of *Octopus vulgaris* (Packard, 1973) has been found to elicit chromatophore expansion in whole parts of the normal patterns (components).

In this paper, we attempt to define the distribution of single motor units, using suction electrodes to stimulate precise nerve bundles to avoid the problems presented by stimulation of the skin surface directly. The results obtained in acute preparations are compared to the patterning of freely behaving animals.

MATERIALS AND METHODS

Animals

Live *Eledone cirrhosa* (Lamark) were obtained from commercial fishermen of Aberdeen harbour and maintained for several weeks in recycled sea water aquaria (10–18 °C) where they were daily fed on live crabs (Boyle, 1981).

Preparation of skin samples

For physiological experiments, the animals were decerebrated without anaesthesia. Pieces of ventral or dorsal skin about 100 cm^2 were immediately excised and pinned, slightly stretched, on a piece of dental wax, epidermal side down. Most of the connective tissue normally lying between the chromatophores and the mantle or arm muscles was then removed. After rinsing in fresh, aerated sea water, the skin preparation was pinned epidermal side up on a cork ring. The ring was placed skin down in a circular Perspex tissue bath where aerated sea water was flowing beneath and above the skin to afford good oxygenation of both surfaces (see Fig. 1). The temperature of the sea water was maintained at 15 °C.

'Denervated' preparations

The left pallial nerve was sectioned proximal to the stellate ganglion in six animals which were subsequently kept in isolation for 7–21 days. Since the somata of the axons

innervating the chromatophore muscles are situated in the brain (Young, 1971), this operation produces degeneration of the chromatophore motor axons, in the stellar nerves and more peripherally (Sanders & Young, 1974). Degeneration of the peripheral end of the axons occurred within 3 days after the operation as shown by the absence of chromatophore response following electrical stimulation of the distal end of the nerve. The absence of functional continuity between the cut ends of the nerve was verified *post mortem* and the non-denervated half of the mantle provided a control.

Recording devices

The tissue bath containing the skin preparation was placed under an inverted microscope, fitted with a fibre optic light source. This allowed a good view of the chromatophores, now lying against the lower wall of the bath (2 mm thick), while the nerve bundles travelling in the connective layers were readily accessible from above for stimulation. With this inverted microscope (Reichert Me F), the chromatophore image (enlarged up to 120 ×) could be projected either onto a ground glass screen or to a ciné or video camera (Fig. 1).

To record the movements of single chromatophores projected on the ground glass screen, a photo-cell was used (silicon photo-diode with integrated amplifier, R. S. Components, Ltd, rise time 30 μs, responsivity 60 mV μW^{-1} cm^2). It was fitted in a black container which could be closed by a selection of lids. Each lid was pierced by a slot of different dimensions and covered by a light diffuser. During the experiments, the cartridge containing the photo-cell was fixed on the ground glass screen, with the slot positioned across the moving edge of a chromatophore. Expansion of the chromatophore across the slot decreased the amount of light received by the photo-cell. The photo-cell signal was amplified and displayed on a pen recorder (Washington 400 MD 2C) together with the stimulus delivered to the nerves.

Alternatively, a black and white video camera monitored the movements of entire chromatophores or groups of chromatophores. The results, recorded on video tape, were analysed from sequential photographs of selected portions of the tape images displayed on a video monitor.

Strictly speaking, both methods used to observe chromatophore behaviour actually record the movements of the pigment cell rather than the length changes in the muscles themselves. However, pigment cell expansion depends directly and uniquely on the length changes in the muscles so that the video recording techniques adequately reflect the number and location of contracting muscles while the photo-cell records the time course of the muscle contraction.

Contractions of the skin muscles (either spontaneous or due to the stimulation) causing movements of the whole preparation often produced irregularities in the baseline. They were minimized by removing most of the dermal layers in which the skin muscles lie and slightly stretching the skin on the cork ring.

Stimulation

Electrical stimulation was delivered to the chromatophores by one of three methods. (1) Stimulation of the small nerve bundles (*dermal nerves*) which run in the dermis separating the mantle muscle mass from the skin and contain axons innervating the

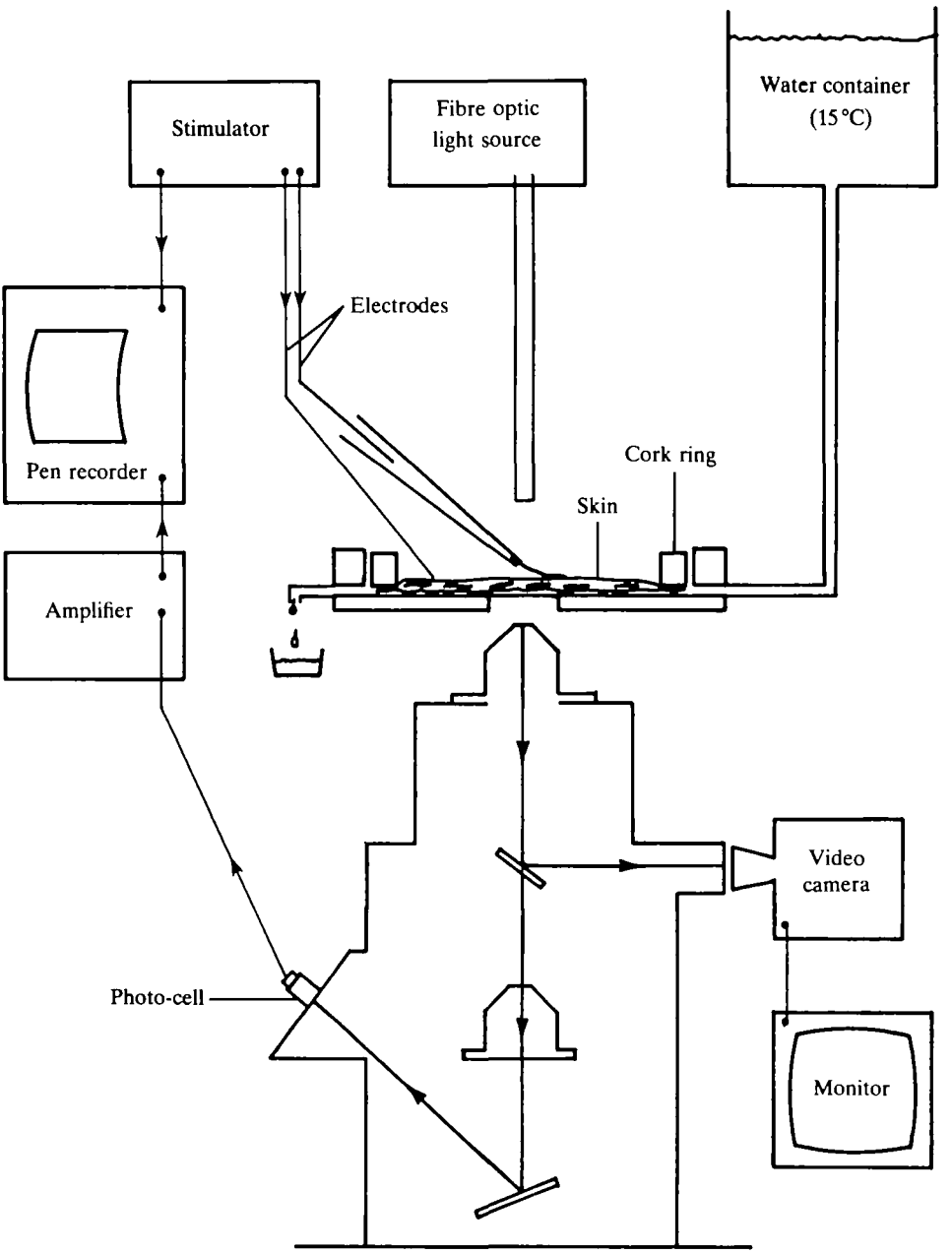

Fig. 1. Diagram of the experimental set-up. The components are not drawn to scale.

chromatophore and skin muscles. Stimulation was applied *via* a suction electrode filled with sea water. Plastic tips of various diameters were used to ensure perfect fitting of the nerve. (2) Direct stimulation of the skin surface either through paired silver wire electrodes (diameter 1·2 mm) or through a micro-concentric bipolar electrode (Clark Electromedical Instruments, diameter of the outer contact 150 μm,

inner contact 25 μm). (3) Stimulation of the chromatophore muscles directly, in 'denervated' skin *via* a glass microelectrode (diameter 10–20 μm).

Square wave pulses were produced by a Devices Isolated Stimulator (Type 2533) driven from a Devices Pulse Generator (Type 2521). When the experiment was video recorded, the stimulus was audible on the sound tract of the video tape and the stimulation parameters were described verbally.

RESULTS

Stimulation pathways

When a dermal nerve bundle in a normal piece of skin was stimulated *via* a suction electrode or when the stimulation was applied to the skin surface directly *via* a bipolar electrode with a supra-threshold stimulus, both the chromatophore and skin muscle contracted. Contraction of the chromatophore muscles caused chromatophore expansion while skin muscle contraction caused skin movements and formation of papillae. By contrast, in 'denervated' skin in which the chromatophore motor axons had had time to degenerate, both stimulation methods failed to trigger chromatophore expansion, although the skin muscle response remained unaffected. This suggests that, with both stimulation methods, the imposed stimulus reached the chromatophore muscles *via* their motor axons. Since the skin muscle response persisted, the axons innervating these muscles presumably have their soma situated more peripherally than the section, probably in the stellate ganglion (Dubas, 1982).

Direct stimulation of the chromatophore muscles in 'denervated' skin was possible only when a glass microelectrode was positioned very close to the chromatophore muscles; even then, considerably higher voltage (up to 50 V) was necessary than in normal skin where 2 or 3 V was sufficient.

Single chromatophore responses

Stimulation of dermal nerves with single supra-threshold square pulses (0·3–0·5 ms duration) resulted in twitch-like contraction of the chromatophore muscle fibres. Within 2–3 h after excision, the rise-time of the chromatophore response was 300–500 ms, depending on the amplitude of the muscle fibre contraction. The expansion-retraction cycle lasted 1000–1500 ms. There was no tonic component and retraction immediately followed expansion (Fig. 2A, fresh preparation).

As the preparations aged, the rise-time often became longer and the amplitude of the expansion smaller. Chromatophores remained expanded for periods of a few seconds to a few hours in the absence of further stimulation. Retraction occurred apparently spontaneously and usually extremely slowly (Fig. 2A, aged preparation).

Facilitation of the mechanical response of the chromatophore muscles was rare. Generally, a single electrical pulse was sufficient to trigger muscle contraction and it produced an expansion of larger amplitude than a train of low frequency stimuli at the same voltage (Fig. 2B). Also, the degree of chromatophore expansion caused by the first pulses of a low frequency train ($F < 5$ Hz) was larger than for subsequent ones. The amplitude of the initial chromatophore expansion was larger because (1) the amplitude of contraction of individual muscle fibres was larger and (2) more muscle

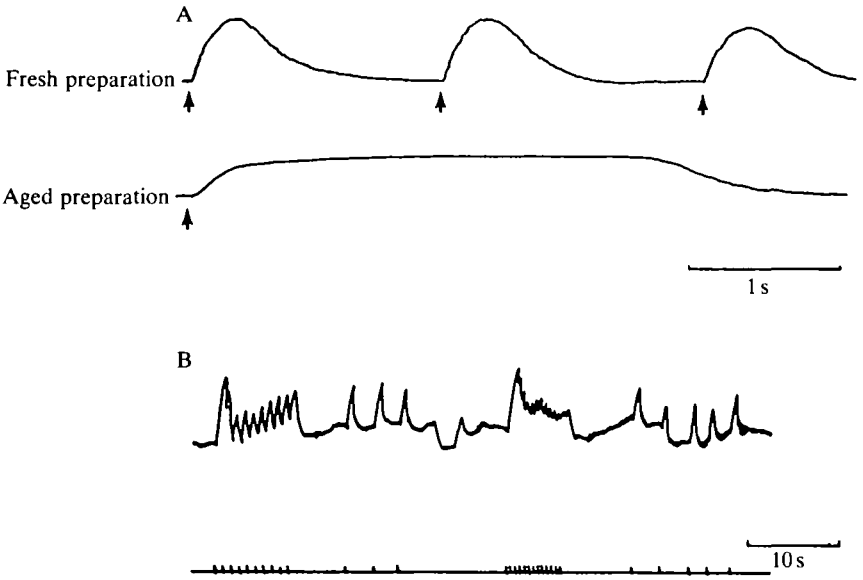

Fig. 2. Photo-cell recordings of mechanical responses of a single chromatophore to electrical stimulation of the radial muscles *via* their motor axons. Each section was recorded from a different chromatophore. (A) Stimulation of fresh and ageing preparations by single pulses (arrows). In the ageing preparation, the chromatophore remains tonically expanded and retraction occurs spontaneously. (B) Low frequency and single pulse stimulation. The upper trace represents the photo-cell response, the lower one the stimulus (duration 5 ms, supra-threshold voltage). The response to the first pulse of a train is always larger than the following ones. Single pulses trigger individual responses larger than those in response to trains of pulses after the first few pulses. The irregularities in the baseline are due to movements of the skin muscles and do not reflect chromatophore muscle activity.

fibres contracted. A change in the quality of the electrode contact was not responsible for this effect since subsequent trains always produced an initially larger expansion.

In fresh preparations, twitches began to fuse at frequencies as low as 2 Hz and summation of the mechanical response occurred. Smooth tetanus was reached at frequencies slightly above 10 Hz. Maximal expansion occurred between 15 and 20 Hz (Fig. 3A). In older preparations, fusion of the twitches appeared at frequencies below 1 Hz.

The twitch/tetanus ratio was fairly low: 1/2 to 1/3 for single muscle fibres (photocell recordings) and 1/3·5 to 1/5 for entire chromatophores (video recordings) (Fig. 3A). The resting/tetanus surface ratio indicated that chromatophores increased their surface by a factor of 15–25 times.

The shape and size of individual expanded chromatophores was modified by variations of the stimulus voltage (delivered to dermal nerves through the suction electrode). At frequencies below 5 Hz, different muscle fibres were brought into contraction at different voltages, presumably when their axon was recruited and the set of muscles of a particular chromatophore was innervated by several axons, not necessarily in the same nerve bundle. Indeed, some chromatophores expanded in response to stimulation of either of two nerve bundles (Fig. 4). Usually, different muscle fibres were recruited by each nerve but sometimes a single muscle fibre responded to stimulation of each nerve. Variations of the stimulus voltage also produced gradation of the amplitude of shortening of individual muscle fibres. This

supports the evidence above for multiple innervation of single muscle fibres.

At tetanic frequencies, variation of stimulus voltage had little effect on the size and shape of the chromatophores since the entire set of muscle fibres apparently contracted maximally when threshold voltage was reached. The behaviour of a chromatophore in response to a range of stimulus voltages, at sub-tetanic (1 Hz) and tetanic (10 Hz) frequencies is illustrated in Fig. 3B.

Chromatophore motor fields

At supra-threshold voltage and tetanic frequency, stimulation of one dermal nerve through a suction electrode produced maximal expansion of all the chromatophores in a discrete area of skin as well as contraction of all the skin muscles of the same area.

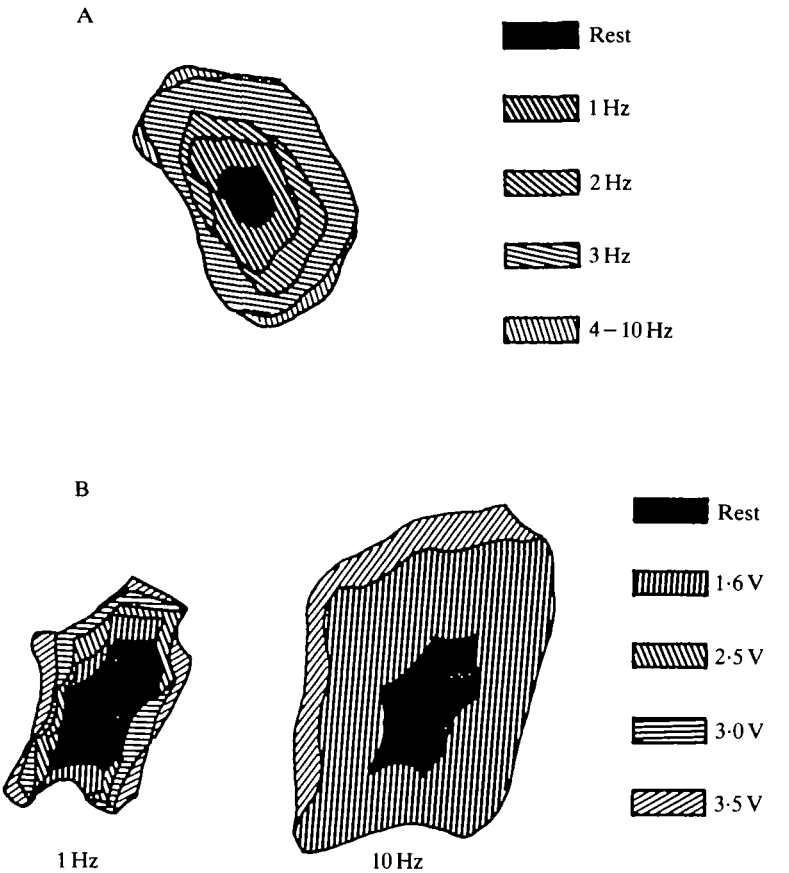

Fig. 3. Chromatophore outlines drawn from the video recordings showing the shape and size of a pigment cell at rest and during stimulation at various voltages and frequencies. A and B represent different chromatophores. The stimulus voltage or frequency were increased in steps and applied for periods of 20 s separated by 30 s of rest. Adjacent non-responding chromatophores were used to permit exact superposition of the outlines. (A) Stimulation at different frequencies and suprathreshold voltage. At frequencies higher than 4 Hz, there is very little increase in chromatophore size. (B) Effect of different stimulus voltages at low (1 Hz) and tetanic (10 Hz) frequencies. At low frequency each voltage step recruits new muscle fibres producing stepwise expansion of the chromatophore. At higher frequency, the chromatophore expansion is nearly maximal and most muscle fibres appear active even at the lowest voltage.

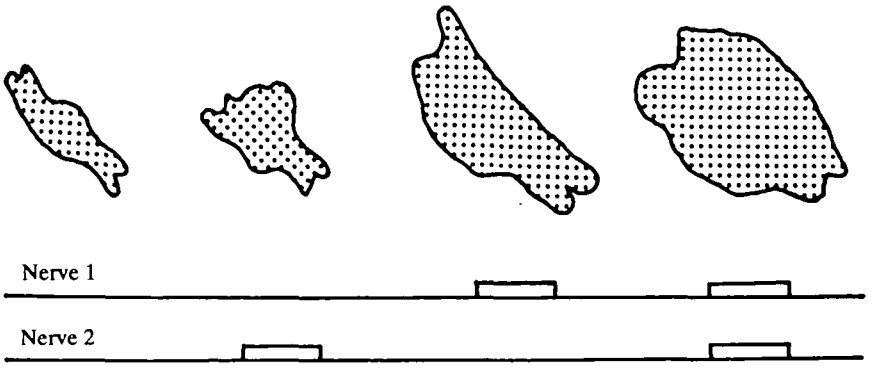

Fig. 4. Sequential drawings of a single chromatophore innervated by axons in two separate dermal nerves. The stimulation was applied individually to two dermal nerves that were clearly not branches of a single bundle. The lower two traces represent the stimulation delivered to each bundle as trains of supra-threshold pulses (pulse duration, 0·5 ms; pulse frequency, 3 Hz; train duration, 60 s).

This area is called the chromatophore motor field of that particular nerve.

Nerve bundles with about 200 axons (estimated from electron microscopy sections) innervated 20 000 to 50 000 chromatophores. Thus, the axon/chromatophores ratio did not exceed 1/100, not allowing for the fact that some axons innervate the skin muscles, some are afferent and some could be inhibitory or have presynaptic action. This suggests that a single motoneurone innervates at least 100 chromatophores and probably substantially more.

Chromatophore recruitment

The area and shape of the chromatophore motor fields were not greatly modified by variations of the stimulus voltage or frequency. However, within the field, the number of expanded chromatophores could be modified by both the stimulus voltage and frequency. Consequently, a decrease in the number of expanded chromatophores due to a drop of stimulus voltage could be compensated for by an increase of pulse frequency. In the following paragraphs, only experiments involving stimulation of dermal nerves are described.

Recruitment through voltage

At stimulus voltages just above threshold, there were patches (not to be confused with the 'patch and groove' arrangement described by Packard & Hochberg, 1977) of expanded chromatophores scattered throughout the entire motor field. Within these patches, the expanded chromatophores were situated in the skin areas surrounding the chromatic units. Chromatic units include clusters of leucophores and iridophores, two types of static chromatic elements, underlying the chromatophores and corresponding to the location of papillae erected in certain patterns (Froesch & Messenger, 1978). With increasing voltage, the isolated patches became linked to each other by areas of

Fig. 5. Detail of a dermal nerve field, showing the distribution of the chromatophores responding to stimulation at 10 Hz and increasing voltage. The stimulus was applied with a suction electrode for periods of 20 s separated by 30 s of rest (A) 0·35 V, (B) 0·45 V, (C) 0·60 V. Magnification, ×3·6.

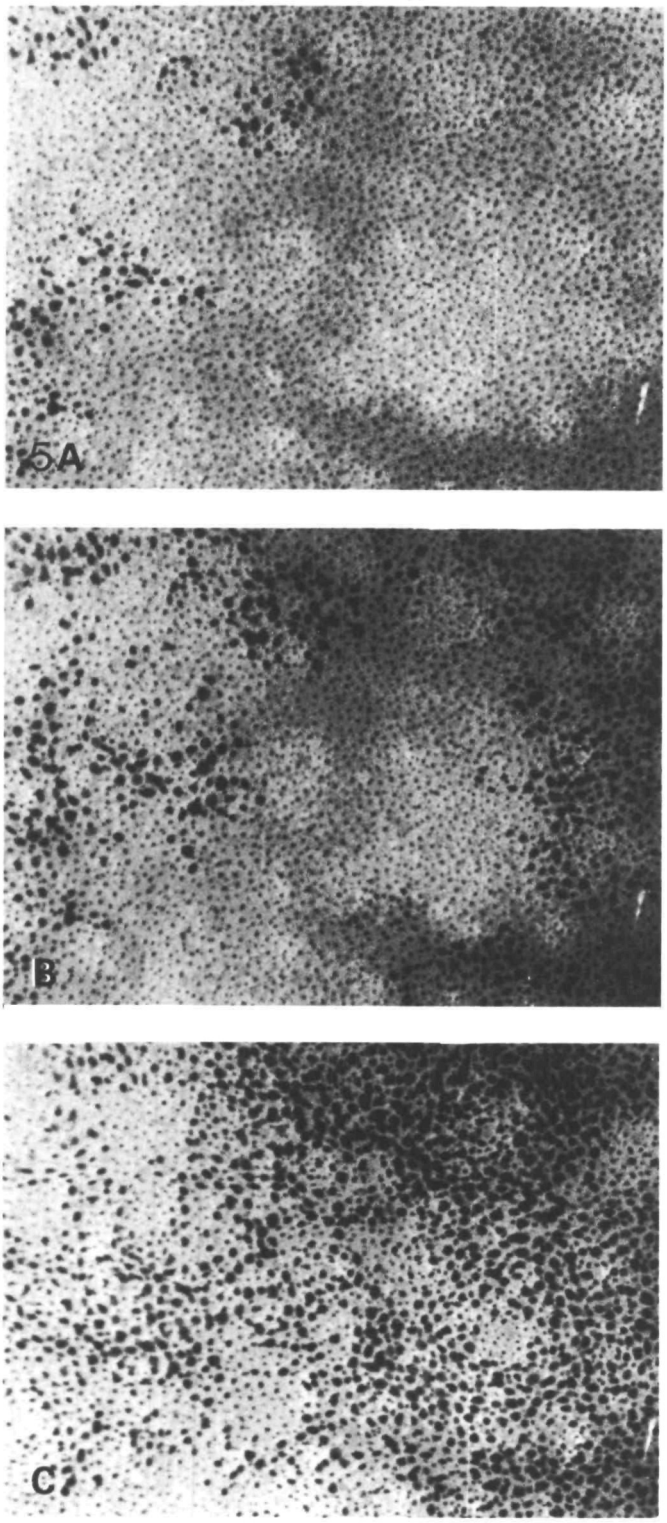

Fig. 5

newly responding chromatophores and, within the patches, the chromatophores lying closer to the centre of each chromatic units were recruited progressively. Only at the highest effective voltage was the centre of the chromatic units finally covered by expanded chromatophores (Fig. 5). Chromatophores recruited at the same voltage rarely formed a compact group (contiguous chromatophores) of more than 20 chromatophores. These effects were characteristic of all dermal nerves or branches of dermal nerves investigated.

Recruitment through frequency

With increasing stimulus frequency, an increasing number of chromatophores could be brought into expansion within the motor field of the nerve stimulated (Fig. 6). At low frequencies, there were only isolated patches of expanded chromatophores. These patches enlarged and merged into each other when the stimulus frequency was raised, rather than becoming linked to each other by new areas of response, as with increases of voltage. Chromatophores recruited by an upward step of frequency were normally scattered among chromatophores responding at lower frequency (Fig. 7). Their distribution did not correspond to the distribution of the chromatophores recruited by an upward step of voltage, for the same nerve. When their muscles were stimulated directly, denervated chromatophores showed the same response as normal

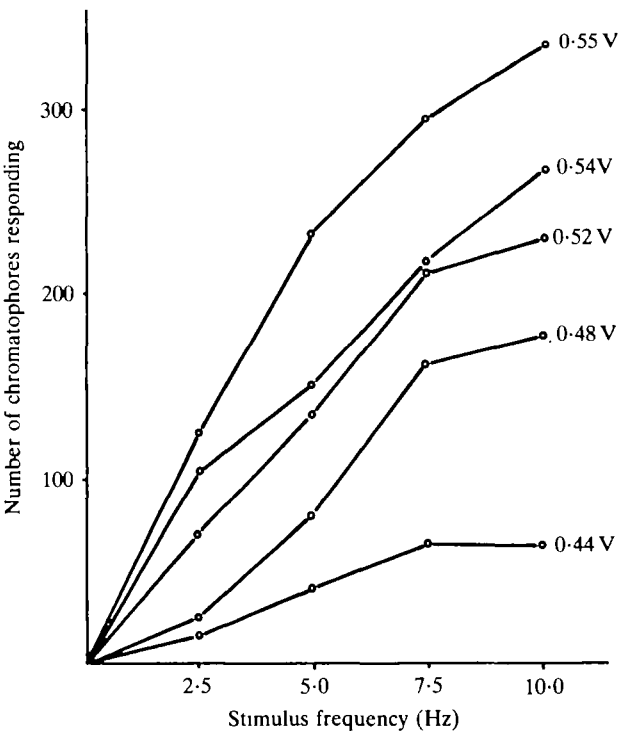

Fig. 6. Number of chromatophores expanding in response to stimulation of a dermal nerve with stepwise increases of frequency and voltage. The stimulation was delivered *via* a suction electrode for periods of 20 s separated by 30 s of rest. Chromatophores were counted from selected frames from the video recordings over an area similar to Fig. 5.

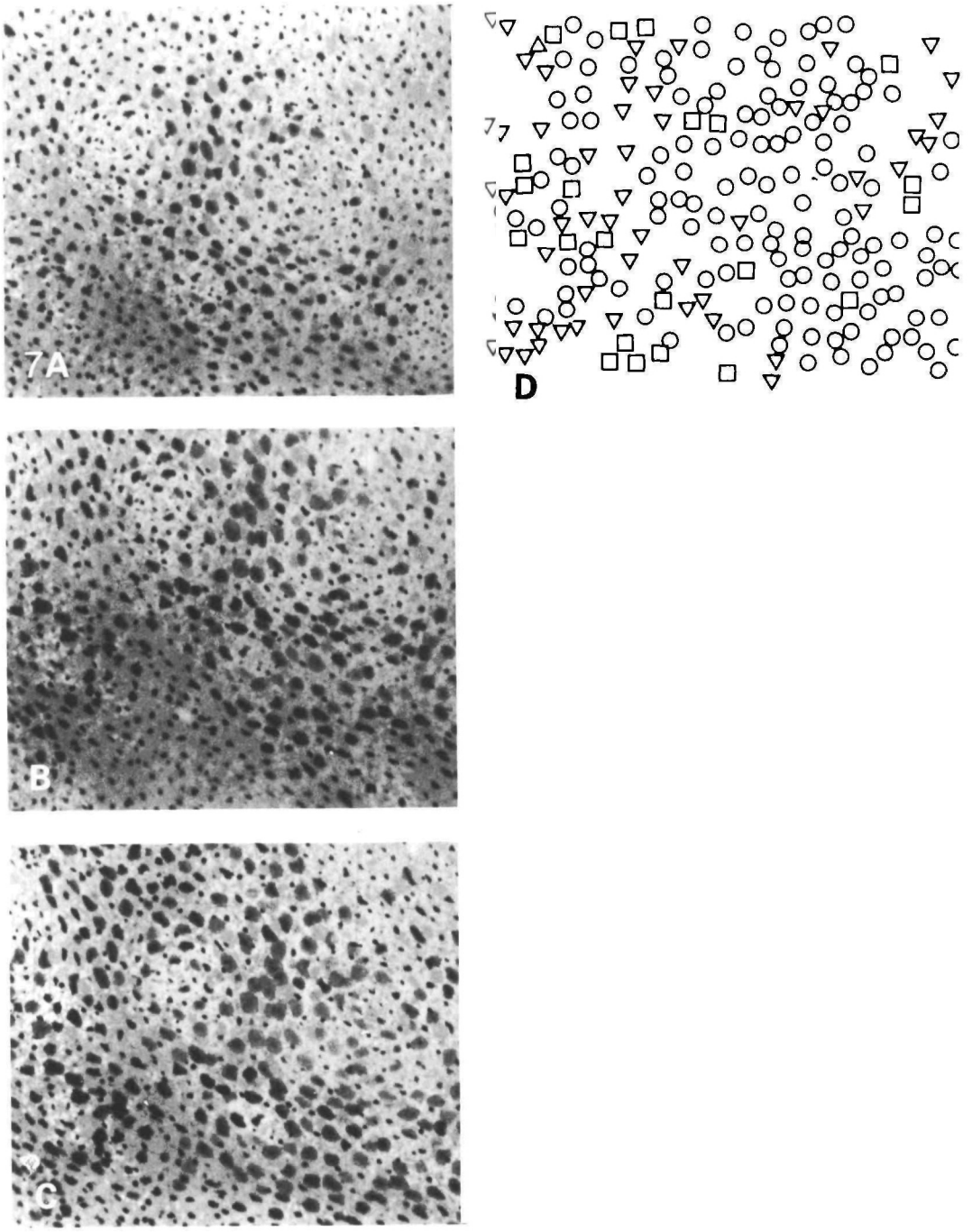

Fig. 7. Detail of a dermal nerve motor field, showing the distribution of the chromatophores responding to stimulation at increasing frequency (supra-threshold voltage). The stimulation was delivered *via* a suction electrode for periods of 20 s separated by a 30 s of rest. (A) 5·0 Hz; (B) 7·5 Hz; (C) 10·0 Hz. (D) Graphic superposition of A, B and C. ○, Chromatophores recruited at 5·0 Hz, ▽ at 7·5 Hz, □ at 10·0 Hz. Magnification, ×4·7.

chromatophores to variations of pulse frequency (Fig. 8). At low frequency, only the chromatophores closest to the glass electrode showed twitch-like expansion, while at higher frequencies, a much larger number expanded.

Some of the chromatophores that could be recruited by stimulation of only one nerve bundle at low frequency responded to stimulation of each of two or more nerves at higher frequency. In fact, at high frequency, all the chromatophores included in the motor field of a dermal nerve were expanded and in the regions where the motor fields of several dermal nerves overlapped, all the chromatophores could be recruited by high frequency stimulation of each nerve bundle.

Observations on live animals

The chromatophore patterns and components of *E. cirrhosa* in aquarium conditions have been identified by Boyle & Dubas (1981). In the following section, the phenomena taking place at the level of small groups of chromatophores are described.

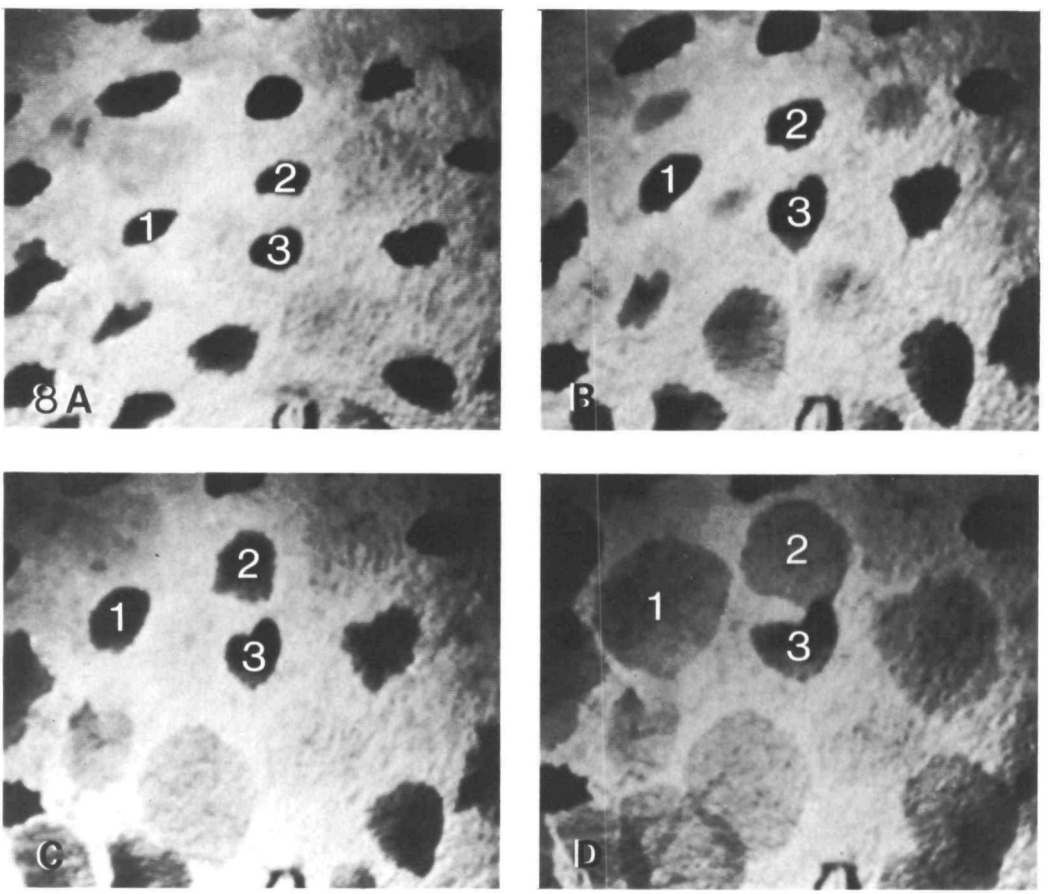

Fig. 8. Photographs from the video recording of a selected area of denervated chromatophores stimulated *via* a glass microelectrode to show that individual denervated chromatophores can also be recruited by variations of the stimulus frequency, without changes of stimulus voltage; (A) rest, (B) 1 Hz, (C) 5 Hz, (D) 10 Hz. Magnification, ×2·5.

In animals at rest and undisturbed, the chromatophores were generally expanded (dressing gown pattern; Boyle & Dubas, 1981). Expanded chromatophores were not visibly twitching but seemed tetanically or tonically expanded. This suggests that the motoneurones fire at relatively high frequency or that a catch mechanism exists.

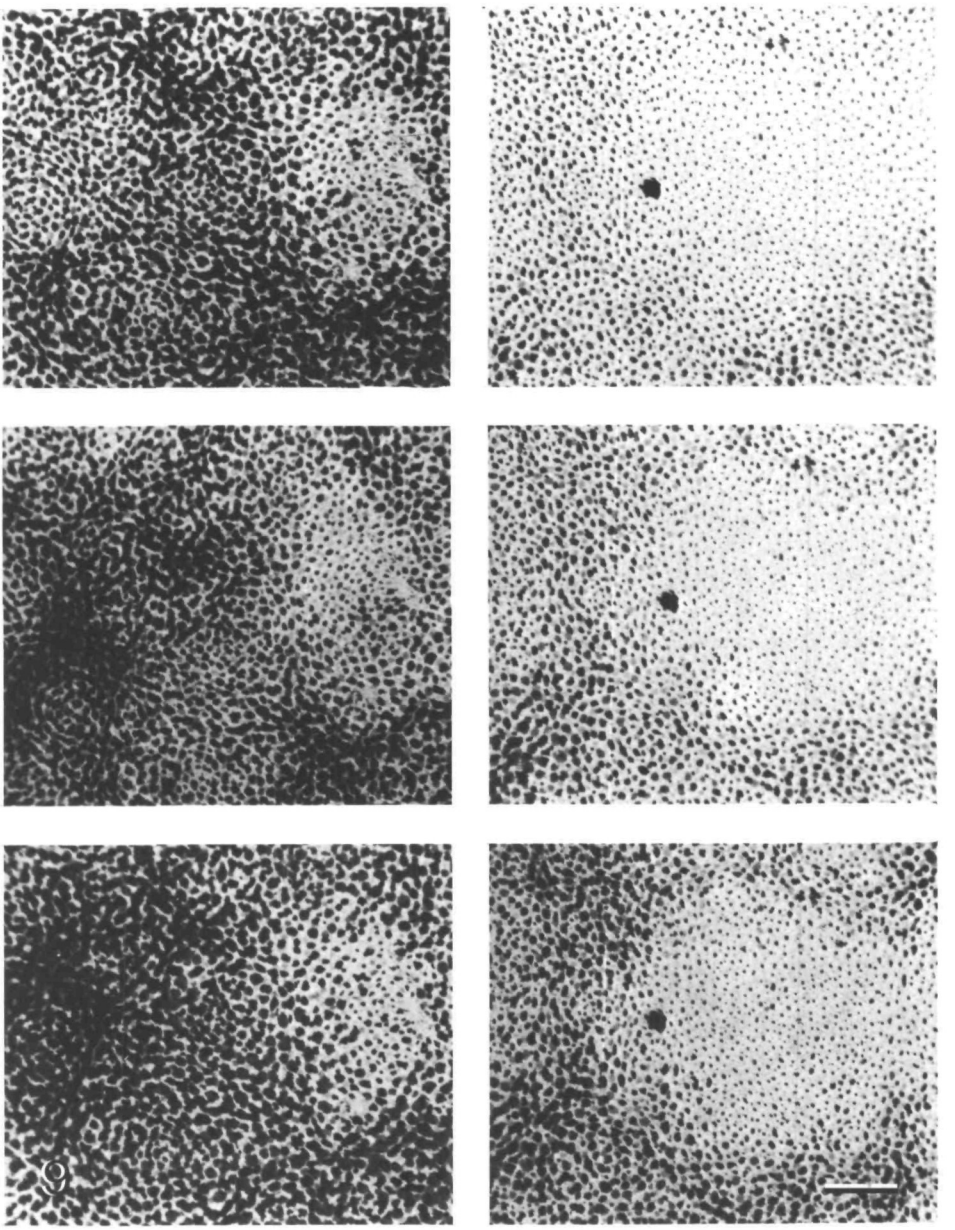

Fig. 9. Close-up photographs of the skin of a freely behaving animal to show phases of the normal patterning. Left and right sequences are of different skin areas. On the left, modification of the size of the mottles. On the right, changes of the intensity of the pattern without modification of the size of the mottles. Scale bar, 10 mm.

The variety of chromatophore patterns that could be observed on the dorsal side of *E. cirrhosa* consisted mainly of a range of mottled patterns made of light, often circular, patches of retracted chromatophores (mottles: this term is used to mean a group of retracted chromatophores, appearing as a single light patch contrasting with a darker background of expanded chromatophores) surrounded by darker areas where at least some of the chromatophores were expanded.

On one hand, the range of mottled patterns was based on variations of the size of the mottles, in other words, on variations of the surface ratio of light to dark areas. This produced a range of patterns of different *coarseness*, from 'all dark' with no light mottles to 'small mottles' where small patches of retracted chromatophores were surrounded by large dark areas of expanded ones, to 'almost light' where the light mottles were very large and had almost merged into each other, leaving very few dark areas of expanded chromatophores. The range of patterns finally ended with 'all light' where the light mottles had completely merged into each other and left no more expanded chromatophores. Serial close-up photographs showed that changes in the size of the light mottles occurred by expansion or retraction of small, scattered groups of chromatophores at the periphery of the mottles (Fig. 9A). The mottles always corresponded to the location of the chromatic units. Thus, a decrease in the mottle size was very similar to recruitment of motor units obtained experimentally by increase of stimulus voltage (compare Figs 9A and 5).

On the other hand, for a given mottle size, the *intensity* of the patterns was varied by changing the number of expanded chromatophores in the dark areas and changing their state of expansion. This produced patterns with a more or less strong degree of expression. Close-up photographs of freely behaving animals showed that graduation of the intensity of the dark areas was achieved mainly by changes of the number of expanded chromatophores and the degree of expansion of individual chromatophores was rarely modified. A given dark area, involved in a low intensity pattern, contained few expanded chromatophores but most of those which were expanded seemed to have reached their maximal size. The same dark area, in a pattern of higher intensity, showed a larger proportion of expanded chromatophores and there was little change in the state of expansion of the chromatophores which were expanded during the pattern of lower intensity (Fig. 9B). This is similar to the effects of increased stimulus frequency obtained during experimental nerve stimulation (compare Figs 9B and 7).

DISCUSSION

Muscle response

In the chromatophores of *E. cirrhosa*, facilitation of the muscle response with stimulus repetition is generally minimal or non-existent, although summation of the contraction occurs. These results are consistent with those of Florey (1966) and Florey & Kriebel (1969) on the chromatophore muscles of *Loligo opalescens*.

Using intracellular electrodes, Florey & Kriebel (1969) have demonstrated that nerve stimulation results only in excitatory postsynaptic potentials in the chromatophore muscles of *L. opalescens*. Local responses might also occur in the mantle muscle of both octopuses and squids (Wilson, 1960). In the chromatophores

of *E. cirrhosa*, the contraction of the muscle fibres is clearly not an 'all or none' event. The observations that the amplitude of the muscle responses diminishes with repetition of the stimulus and can be graded by variations of the voltage both suggest that the contraction is mediated by graded potentials, rather than 'all or none' spikes.

With stimulation of the dermal nerves there is no evidence for inhibitory innervation but this does not exclude presynaptic inhibition. Indeed, such presynaptic control would not have a 'retracting' effect but would presumably prevent the excitation reaching the muscle fibres and thus could easily pass unnoticed during the experiments. Direct inhibitory innervation has been ruled out by Florey & Kriebel (1969), who recorded intracellular changes of potentials, but their method does not exclude presynaptic effects.

Motor units

Since electrical stimulation was applied to the dermal nerves which contain several chromatophore motor axons, it was difficult to determine how many motor units were stimulated at any one time and to determine the distribution of single motor units. On the other hand, since the dermal nerves were stimulated before they branch (Dubas, 1982), it was certain that entire motor units were stimulated and not parts of them.

Thus, the chromatophore motor units of *E. cirrhosa* seem to have the following characteristics: (1) a single chromatophore and individual muscle fibres can be part of several motor units; (2) a single axon innervates a large number of chromatophores (and within a single chromatophore, several muscle fibres), not all clustered together but scattered over a large part of the chromatophore motor field of the dermal nerve considered; (3) the distribution of a single motor unit is not coincident with a single chromatic unit but chromatophores innervated by a single axon appear to be located at similar distances from the centre of the chromatic units they lie in. Points (1) and (2) are in agreement with the results obtained by Florey (1969) although the chromatophores innervated by a single axon are more clustered in squids.

Since the distribution of the motor units coincides with the distribution of the mottles shown during normal patterning, it is clear that the motor units of the dorsal skin of *E. cirrhosa* are not random but correspond to parts of the normal patterns (innervation type C, Maynard, 1967). Thus, the chromatophore muscle fibres which are innervated by a single axon are involved in a particular part of a pattern. Assuming that a single axon needs to be active to trigger muscle contraction, the number of axons innervating a single muscle fibre is perhaps representative of the number of patterns in which that particular fibre plays a role.

Pattern control

Since the distribution of the motor units is non-random, the repertoire of patterns is determined to a large extent in the periphery, by the morphological layout of the motor axon branches among the chromatophores. Thus, as far as the mottle patterns are concerned, the role of the brain seems to be to coordinate the activity of the patterned motor units to produce gradation of the coarseness or intensity of the patterns.

Comparing the present experimental results with patterning in intact animals, it appears that the size of the light mottles is regulated centrally by recruitment or

inhibition of motor units, possibly by lateral inhibition mechanisms. On the other hand, the experimental results suggest that changes of the intensity of the patterns may be brought about simply by variation of the frequency of the nerve discharge since this produces recruitment of chromatophores in the areas where some chromatophores are already expanded. The colour patterns are thus comparable to other neuromuscular systems where the intensity of the contraction is graded both by the frequency of the nervous discharge and the number of motor units recruited.

Recruiting effect of frequency

Our evidence suggests that the recruiting effect of frequency is due to characteristics of the muscle fibres rather than the nervous system. So far two hypotheses can be brought forward. (A) Two types of neuromuscular systems may coexist; a non-facilitating and a facilitating system. The non-facilitating system, demonstrated by the present results, responds with maximal contraction even to a single stimulus. A facilitating system, for which direct evidence has not been obtained yet, consisting of separate axons, may produce visible contraction of the muscle fibres only above a threshold frequency. Since multiple innervation is the case, these muscle fibres may be the same as those of the non-facilitating system, being able to show two types of responses, depending on the type of axon firing. (B) Alternatively, the presence of electrical links between the muscles of adjacent chromatophores may allow the depolarization evoked by nervous activity to invade the coupled muscles. Florey & Kriebel (1969) have presented intracellular evidence that the muscle fibres of a single chromatophore are linked by low resistance pathways. Froesch-Gaetzi & Froesch (1977) have argued, on the basis of morphological evidence, that such links also exist between the muscle fibres of neighbouring chromatophores. Further investigations with intracellular electrodes are required to determine whether one or both possibilities occur.

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