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ACTIVITY OF INTERNEURONES IN THE ARM OF *OCTOPUS* IN RESPONSE TO TACTILE STIMULATION

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INTRODUCTION

This is a preliminary account of recordings from the nervous system of the arm of *Octopus*. The results are incomplete, but are presented at this stage for two reasons. First, their relevance to recent work on the anatomy of the arm nervous system of *Octopus* and on its tactile learning system, and on the cephalopod CNS in general. Secondly, a technique has been developed for obtaining reliable recordings and a long-lived and easily managed preparation. Lack of this has hindered electrophysiological investigation of the cephalopod C.N.S. in the past.

METHOD

(1) *Preparation of the animal*

Octopus vulgaris of both sexes, weighing between 400 and 700 g., were obtained from the Bay of Naples in July 1965. They were kept and fed for some days in the laboratory before use.

Isolated arms of *Octopus* have a short life, enough for some behavioural studies but inadequate for electrophysiology (Rowell, 1963). The cause of death is lack of blood circulation. It is well known that when the arm is injured there is vasoconstriction of the proximal vessels, which prevents excessive loss of blood. This also occurs distally. If an isolated arm or one still attached to a living animal is dissected, the blood vessels are inconspicuous, the sinuses are empty, and what blood is visible is whitish (deoxygenated). As will be shown later, this is not the functional condition of the vessels.

Attempts at perfusion of the blood vessels were unsuccessful. A solution of the same ionic composition as the blood, made isosmotic with sucrose, retarded death for some time but reflex responsiveness was poor. The tissues became oedematous, possibly due to the absence from the perfusing fluid of the large concentrations of protein present in cephalopod blood. Maturana & Sperling (1963) successfully perfused the isolated statocyst with aerated sea water. This gives gross oedema when used with the whole animal, but the statocyst is enclosed in cartilage, which may prevent too great hydration. However, the primary receptors are less sensitive to the state of the circulation than is the C.N.S., and it is also likely that the vessels to the statocyst are less well provided with shut-down muscles than those to the arms. As far as the arms are concerned there appears to be no simple substitute for the natural circulation.

If, however, the intact animal is used there is major difficulty in immobilizing even a portion of it sufficiently to apply electrodes. Lettvin & Maturana (who also found

that the integrity of the circulation was essential) solved this problem by drastic surgery (personal communication). They studied the optic nerve. The entire web of arms was cut off, the body nailed to a board, the siphon immobilized by having a glass rod passed down it, and finally the orbital musculature was sewn to a metal frame. This approach is inappropriate for the arm, as the muscles are strong enough to tear the arm from around any nails or clamps. If the whole animal is to be used it is necessary to abolish the motor output to the arm. If this is done by cutting the axial cord, the blood vessels of the arm contract and death follows. The other method available for preventing motor output is to abolish it at the brain. A suitable lesion was found to be the removal of the entire supraoesophageal ganglion (Fig. 1 A). An animal so operated

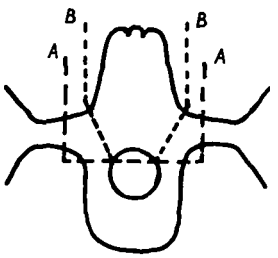

Fig. 1. Diagrammatic transverse section through the brain of *Octopus* showing the two lesions, *A* and *B*, used to produce immobility in the whole animal. All material above the dotted lines is removed. Lesion *A* is the complete removal of the supra-oesophageal ganglion, and the disconnexion of the optic lobes from the suboesophageal ganglion; lesion *B* removes most of the supraoesophageal ganglion, but allows some connexion between the optic lobes and the suboeosphageal ganglion to remain. Further explanation in text.

upon has well-known characteristics (see Wells, 1962), including the posture in which the arms are all reflexed around the body and permanent blanching of the chromatophores. It cannot feed, but lives for some weeks without food. For the purpose of arm physiology it has many desirable features. Respiration and circulation continue normally; the power of aggressive jetting of ink and water through the siphon is lost; there is greatly reduced spontaneous movement, the animal mostly just sitting still; and under stress, e.g. when handled, it shows the phenomenon known as 'sticky suckers', in which the animal has difficulty in letting go of objects in contact with the suckers. For this reason, it cannot walk away readily when being set up in the experiment.

The procedure was to remove the supraoesophageal ganglion (though not the optic lobes) under urethane anaesthesia (1% in sea water), suture the wound and allow 2 days for recovery. This interval does not seem to be strictly necessary, and on occasion animals were used the same day. For experiments, the animal was transferred to a lead-lined wooden trough, 125 cm. long, 30 cm. broad and 10 cm. deep. Sea water flowed in at one end of this trough and was aspirated from the other by a filter pump. A wooden board 25 cm. square was held to the floor of the trough by lead weights. The octopus was put on the floor of the trough to one side of the board, and one arm was grasped and extended over the board. It was secured in this position by two stainless-steel spikes inserted through the dorsal musculature in such a way as to avoid major nerves and blood vessels. The animal struggled during this procedure, but much less than an intact one, and ceased to struggle as soon as it was over. A wooden strip

with a notch in it to accommodate the arm was placed across the trough, to separate the octopus from the working space. More boards were placed across the trough over the octopus and weighted. Thus provided with a narrow dark space in which to sit, the octopus remains quiet and peaceful (Fig. 2). The arm was usually positioned so that the lateral surface was horizontal, and then the lower of the two rows of suckers grasped the base board and the upper row remained free. The free distal end of the arm usually bent back on itself and wrapped round the fixing spikes. The skin and musculature of the lateral surface were split longitudinally for a few suckers' distance, and the axial cord was exposed. If this is done swiftly with sharp instruments the animal does not tear itself free, and it is prevented by the boards from reaching the operation site with the other arms. The free distal end attempts to grasp the instruments, but is small enough to be easily deflected.

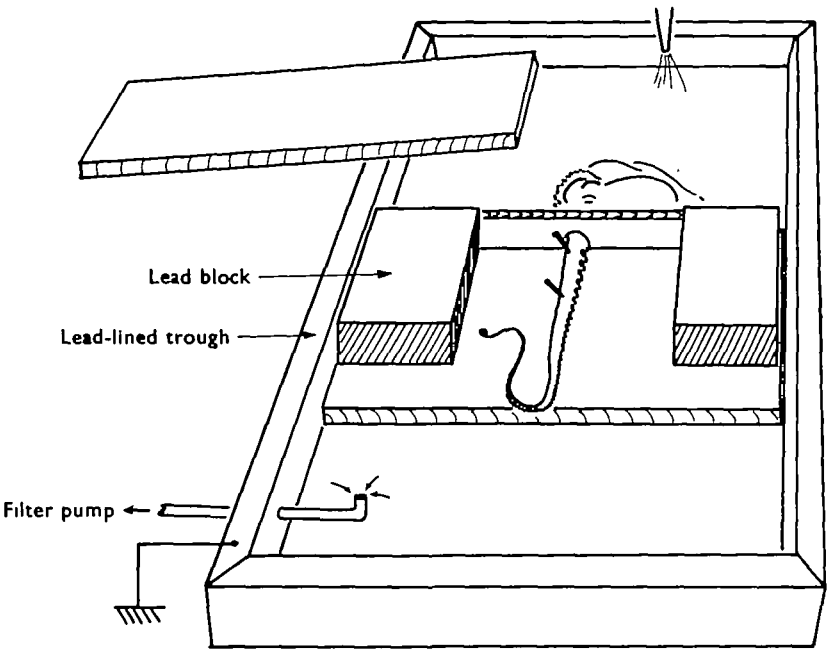

Fig. 2. Diagram to show the arrangement for recording from the octopus in a shallow lead-lined trough of running sea water. The board which normally covers the animal is shown lifted.

When the arm is opened up the blood vessels are all constricted and they remain so for some time. Consequently during the first 40 min. or so there is a fall-off in reflex responsiveness and (when appropriate, see below) chromatophore colour. If left in peace, the vessels then expand again to what is presumably their normal state, very obvious, filled with dark-blue blood, and the venous sinuses fill and pulsate. From this time onwards responsiveness and colour begin to return, and thereafter the preparation will behave well and live for at least ten hours. If additional major lesions are made the process is repeated, though less markedly if the animal is not handled afresh. If, however, a major blood vessel is accidentally broken after the circulation is restored catastrophic bleeding occurs. A certain number of nerves are unavoidably cut when exposing the cord, and the areas innervated by them remain permanently white and

flaccid, but apart from this the animal is quite normal and can be returned to its tank at the end of the day and used again. The operated arm is functional. If the arm is amputated, it shows the full range of reflexes shown by an arm amputated from a fresh animal (Rowell, 1963).

For some purposes it is advantageous to modify the brain lesion so that some connexion remains between the optic lobes and the suboesophageal ganglion (Fig. 1 B). This leaves the animal rather more difficult to handle. It responds to some optic stimuli, and is considerably more mobile; it swims readily, and can jet both water and ink, though not with the usual precision. When set up, however, it is equally placid and has the advantage that the chromatophores, instead of blanching, remain permanently expanded. This is very useful both for studying the chromatophore innervation and also because the chromatophores are a most sensitive indicator of the functioning of the blood circulation, and give visible evidence of deterioration long before anything else.

The exposed nerve cord was stained lightly with methylene blue in sea water. The staining produced was variable, sometimes taking mainly to cell somata, sometimes to connective tissue, but always helped. It is quite essential if peripheral nerves are to be cut, as the cut end contracts and is lost in the mesh of connective tissue unless differentially stained. The stain appeared to have no adverse effects on function.

(2) *Recording methods*

Recordings have to be made under sea water; the nerves are not long enough to lift clear, and die if stretched or allowed to dry. Insulated steel micro-electrodes and metal-filled micropipettes occasionally recorded transient bursts of activity at low amplitude, but were usually ineffective. Glass micropipettes broken down to a coarse tip of 10–20 μ and filled with sea water were also sporadically effective, but were enormously improved if connected to a suction line. These electrodes were successful either if applied to the surface of the nerve cord or if inserted into its thickness. Often a unit could be found by ear while probing with the electrode disconnected from the suction line; when a favourable site was found, the line was reconnected, and as the pressure fell the recording would gain several times in amplitude. When firmly sucked on, the electrode recorded the same units for hours on end. Without suction the units gave small records and were almost immediately lost, presumably due to sea water leaking between the tip and the active elements. Coarser pipettes were also used as orthodox suction electrodes on the cut ends of sucker nerves. The amplitude of the record obtained is entirely dependent on how tightly the nerve fits the pipette. The nerves running from the axial ganglia to the suckers were cut at the junction with the ganglion, and the ends were sucked into pipettes for recording and stimulation.

Two input arrangements were used; a cathode-follower-connected electrometer tube, and a field-effect transistor with an input impedance of 20 MΩ. The former gave a rather higher S/N ratio, but the transistor stage had other advantages, especially freedom from microphony, and was more often used. The largest potentials recorded with this preamplifier were about 700 μV., and the general run were 200–300 μV. in amplitude. This is acceptable considering that virtually all units recorded were afferent fibres, the great majority of which are $< 6\,\mu$ in diameter, with a very few reaching 12 μ (Young, 1965).

RESULTS

(1) *The peripheral nerves*

It is known (v. Uexküll, 1894; ten Cate, 1928; Rowell, 1963) that these are mixed nerves. Only the afferent fibres were investigated, i.e. the nerve distal to the cut. In all preparations some response to tactile stimulation of the sucker could be heard via an audioamplifier, and when the nerve was a tight fit in the pipette good records were obtained. There was no recordable resting activity, as was also found by Maturana & Sperling (1963) in statocyst afferent fibres.

Recordings were always dominated by a few axons in each nerve responding to tactile stimulation of precisely localized points on the sucker rim. It is presumed that these axons are much larger than the remainder. When magnified, the edge of the sucker rim is seen to be made up of many small segments, giving it a 'cogged'

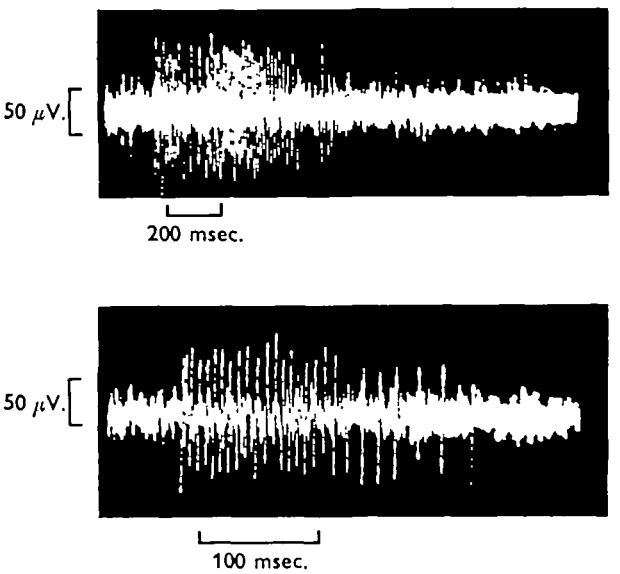

Fig. 3. Recordings from the cut sucker nerve at two different sweep speeds of the response to tactile stimulation of a small discrete area of the sucker rim, presumed to contain a receptor sensitive to mechanical deformation. Further explanation in the text.

appearance. The effective stimulus was mechanical deformation of a particular segment. The deformation can be from any direction, and the response varies little with intensity, provided it is above threshold. The segment can be gently pressed, nipped with forceps, jabbed with a needle, or slowly flattened. To all these the response is a short burst of *c.* 200 msec. length. Where a single unit can be identified with certainty, it fires about 20–40 times in such a burst (Fig. 3). The units are not specially sensitive, not responding to a drop of water dropped on the sucker rim from a height of 1 cm. They do not respond to dilute acetic acid of a strength which gives a violent behavioural response. They are phasic, habituating rapidly to sustained distortion, and tire quickly: after 6–8 stimuli at 1 sec. intervals they do not respond at all, and require a minute or more for recovery. It is suggested that the receptor is sensitive to distortion, and is located below the surface of the skin. Other tactile receptors could

be heard, though not seen on the CRO, and some were localized on the surface of the infundibulum. None of those found were located on the acetabular surface. (Morphological terms after Rossi & Graziadei, 1958, see Fig. 4.)

All receptors localized on the rim were within the area innervated by the motor fibres carried in the same nerve. Stimulation of the nerve using the same electrode gave local contraction of the rim muscles and expansion of chromatophores over a discrete area (see Rowell, 1963). The sensillum was always located in this area.

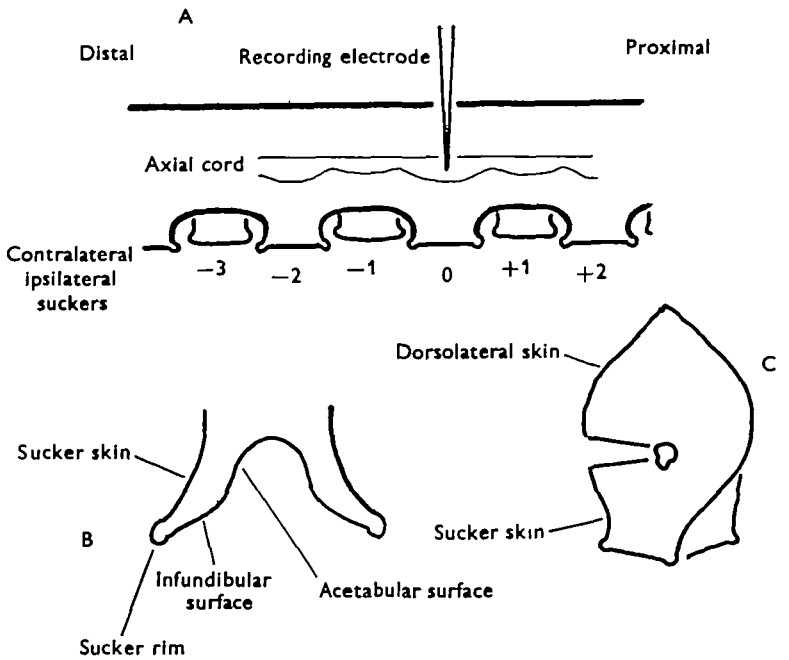

Fig. 4. Diagrams to show the nomenclature used in describing the anatomy of the arm and the results of recording. A. Lateral view of the arm. A recording electrode is placed on the exposed axial cord. The sucker which is innervated by the axial ganglion nearest to the electrode is called 'sucker 0'. Suckers proximal to this are numbered '+1', '+2', etc., and distal suckers are numbered '−1', '−2', etc. As the suckers are arranged in two rows, it follows that the row which is ipsilateral to the recorded sucker has even numbers, the contralateral row odd numbers. B. Longitudinal section through a sucker, showing the terms used for different areas of its surface. C. Transverse section through an arm at the level of an incision down to the axial cord as for recording, showing the terms used for the different areas of its surface.

(2) *The neuropile of the axial ganglia*

The axial ganglia are masses of neuropile attached to the ventral surface of the axial cord, one to each sucker. The cell bodies of their interneurones lie peripherally around the ganglion. Bundles of fibres can be seen to leave the longitudinal tracts of the cord and run into the neuropile of the ganglia, and each ganglion gives rise to the peripheral nerves, about twenty-eight in all, which serve the arm and especially the suckers.

A few recordings were made from the axial ganglia, and the following units were well enough established for description.

(*a*) Units responding to tactile stimulation of the skin of the ganglion's own sucker. The sensitive area includes the sucker rim and cup, and all the skin dorsal to the

sucker as far as the midlateral cut exposing the ganglion. There was no response to stimulation of the adjoining proximal or distal suckers or arm skin, nor from elsewhere on the arm. The units adapt quickly, giving bursts of not more than 50 impulses to a maintained stimulus. Threshold was relatively high (Fig. 5*a*).

(*b*) Units similar in behaviour to (*a*), but with a sensory area restricted to suckers −1 and −3; that is, to the two adjoining distal suckers of the contralateral row (Fig. 5*b*).

(*c*) Units similar to (*a*), but having as sensory area only the immediately adjoining sucker, either proximal or distal (i.e. suckers −1 or +1) (Fig. 5*c*).

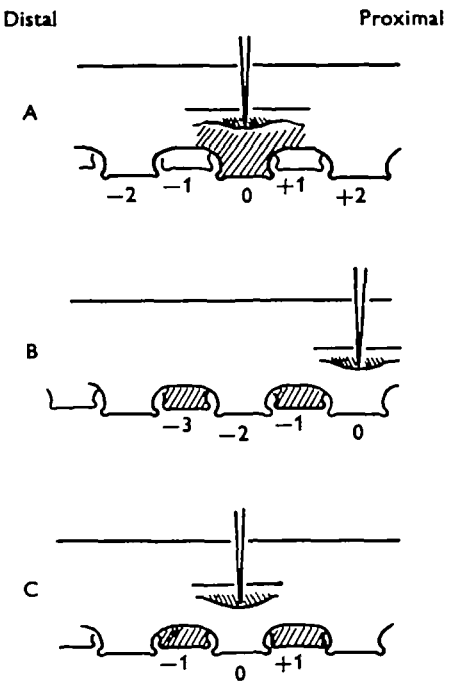

Fig. 5. Diagram to show the receptive fields of 3 different types of units responding to tactile stimulation of the sucker skin found in the neuropile of the axial ganglion. The receptive fields are shaded. Further explanation in the text.

(*d*) Units which respond to movement, either passive or active, of the sucker innervated by the ganglion. They presumably derive their input from muscle receptors (Graziadei, 1965). They are phasic, firing only at a change in sucker position or contraction of the muscles.

(*e*) Large units which are probably motor cells running to the sucker musculature. Insertion of the electrode into this site gave a general contraction of the muscles of the entire rim, and a long discharge. Thereafter that unit only fired when the rim muscles contracted. Electrical stimulation at this site produced general contraction of the rim musculature.

(3) *The longitudinal tracts of the dorsolateral cord*

The main part of the axial cord is composed of longitudinal tracts running between the brain and the axial ganglia. These tracts seem to be without cell bodies or synapses. At least the majority of these axons are interneurones. There has been no unequivocal demonstration of primary sensory or final motor pathways in this region, though Young (1965) considers it probable that the chromatophore motor fibres run direct from the brain to their muscles. All units recorded in these experiments were apparently interneurones.

The majority of recordings were made from the longitudinal tracts (about forty good sites were investigated), partly because of their ease of access, and also because of the interest in what level of information is supplied to the brain, in view of the animal's ability to learn only certain types of discrimination from sensory inputs to the arm (Wells, 1964, and previous papers). The records obtained are in general characterized by the following:

(i) It is rare to get responses consisting of only one or a few units—usually many different units are present. This suggests that finer electrodes would be desirable for future work, if single neurones were to be investigated.

(ii) 'Spontaneously' active units are rare. However, the preparation of the animal is specifically designed to reduce spontaneous motor activity.

(iii) Motor units are rare, even when the arm is stimulated into prolonged motor activity. It may be that the motor fibres run in an area rarely sampled, or perhaps relatively few fibres carry motor signals.

(iv) There is no apparent representation of movements of suckers or of the distal tip of the arm.

(v) There is a very detailed, though highly integrated, representation of tactile sensory input. This representation has some peculiar features: it habituates readily to even complex repetitive patterns of stimulation, but recognizes small deviations from this pattern, and certain expected inputs, especially those derived from tactile stimulation caused by spontaneous movement of the arm, are not represented at all. No recording of propioceptive information of any sort was obtained, though this is present at the level of the axial ganglion.

Examples of specific recordings illustrating these and other points are given below.

(*a*) *Sensitive areas: spatial localization of stimulation*

When activity was elicited at a recording site by tactile stimulation, the arm was mapped into those areas:

(A) that gave an apparently identical response, and

(B) those that gave either no response or a demonstrably different one.

As category (A) cannot be satisfactorily defined, such a description will attribute to the units in question the least flattering estimate of their discriminatory power. It must be remembered that there is certainly greater precision of spatial representation in the afferent interneurones than this method shows, if only because it is known that only a very small number of the responding interneurones are sampled at a single site. The following is a list of the sensitive areas served by specific units or groups of units, in order of approximate size.

(*a*) The rim only of a single sucker.
(*b*) Outer skin of a single sucker.
(*c*) The outer skin, or the rims of two adjacent distal or proximal adjacent suckers.
(*e*) Sucker rims and lateral skin of all suckers of the distal part of the arm.
(*f*) All suckers of the distal arm.
(*g*) Lateral and dorsal skin of the distal arm, but not suckers nor ventral skin.
(*h*) All skin and all suckers of distal arm.
(*i*) All suckers and all skin of proximal arm.
(*j*) Entire arm—ipsilateral suckers only.
(*k*) Entire arm—all suckers.
(*l*) Entire arm—deep prick to musculature, no response to skin.

All these units have similar characteristics: they are phasic, and habituate or fatigue rapidly with repeated stimulation. As a rule, fibres responding to stimulation proximal to the recording site (i.e. propagating centrifugally) adapt slower than those from distal areas and their spikes are of smaller amplitude, suggesting fibres of smaller diameter.

Fig. 6A shows the patterns of activity recorded at a single site when various suckers of the distal arm were prodded with a blunt needle on the infundibular surface. Despite so imprecise a stimulus it can be seen that there is a clear common pattern in successive presentations at any one site, but marked differences between the signals received from different areas. The input from sucker -4 differs from that from -5, sucker -5 is different from sucker -7, -7 and -9 are indistinguishable and probably the the same, -30, is different from any of the other groups. All three groups differ from the response obtained to a touch on the lateral skin at the level of sucker -5 (Fig. 6B).

The differences seen are of several sorts: spike size, length of burst, and temporal patterning. The first indicates different units. The second might be explained on conduction velocity differences, the more distant areas giving a more spread out response, but the composition of the burst with regard to spike size makes it unlikely that this is the only explanation, and it would imply a conduction velocity (250 msec. for 10 cm.) of only 40 cm/sec., which is rather low (compare Burrows, Campbell, Howe & Young, 1965). The difference in temporal pattern is clearly seen by comparing the responses from the suckers, which show only one peak of activity, with that from the lateral skin, which regularly produces two peaks.

(*b*) *Sensitivity of tactile interneurones*

There is a considerable range in threshold. Most units were stimulated by gentle stroking of the skin with a needle. Some did not respond except to blows or pinches of considerable force, enough to produce behavioural response from the other arms. Some were extremely sensitive; for example, one large unit which responded to stimulation of distal sucker rims gave a definite burst to a drop of water falling 1 cm. on to the water surface 15 cm. away from the suckers, which were themselves 0·5 cm. under the water surface.

Graded response to increasing intensity was well illustrated at a site which responded to stimulation of two adjacent ipsilateral suckers only. Usually this produced a burst

from three units with different spike sizes. With higher levels of stimulation more spikes occurred. At lower levels two of the units dropped out in turn, leaving only one, which also gave the largest amplitude spikes. With a very gentle touch it was possible to get a response consisting only of one action potential in this single unit.

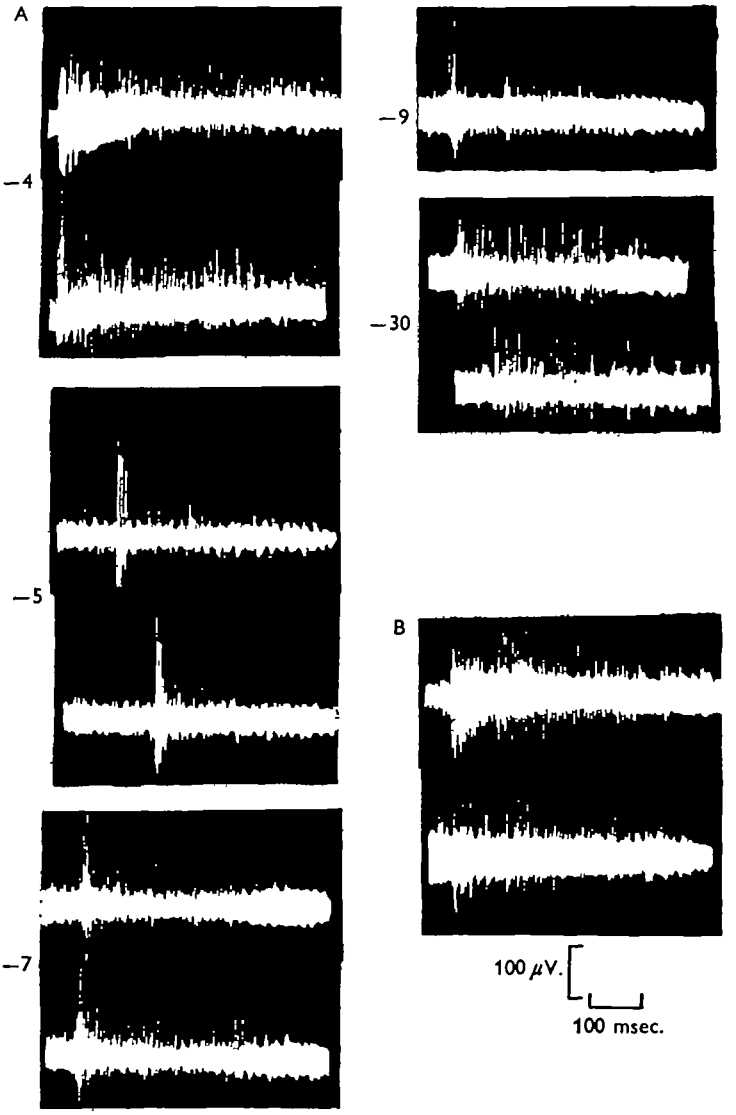

Fig. 6. Recordings from a site in the dorsolateral longitudinal tracts of the axial cord. Each picture shows the response to momentary tactile stimulation of an area of skin distal to the recording site. A. Stimulation of sucker skin: sucker −4, two records; −5, two records; −7, two records; −9, one record; and −30, two records. B. Stimulation of dorsolateral skin at the level of sucker −5, two records. Records for two sites (sucker −9, and dorsolateral skin) have been retouched on the lower edge.

(*c*) *Habituation and recognition of pattern in tactile interneurones*

The units are fast-adapting and phasic, responding most to a change in input, rarely to a sustained stimulus. In addition they habituate rapidly if repeatedly stimulated. If a group of suckers is allowed to grasp a glass rod or a finger, there is an initial burst of activity in the interneurones on contact and as the suckers seize hold. Thereafter, if the object is not moved, there is little or no further activity, even though the suckers may feel the object over for some time, and eventually relinquish their grip and possibly push the object away. If the object is moved while the suckers still have a grip of it, there is a new burst of activity, and the suckers usually tighten their grip. If this movement is repeated, there is a synchronous burst at every movement, provided the interval is long, say not less than 20 sec. If the movements is repeated regularly at 1 sec. intervals, the response ceases after about ten to twenty trials, though the grip is retained. If the direction of movement is now altered, however, the bursts start again at once, and to a casual inspection it looks as if they are composed of the same units as responded initially. If this is so, the initial waning of the response is due to habituation, not to fatigue. A sequence of regular movements of one type until waning has taken place, broken by one or two of another type, and then reverting to the first type produces little or no response at the second presentation. If, again, the same units are involved, this would imply a mechanism of pattern 'memory' at the interneurone level.

(*d*) *Maintained activity in interneurones after initial stimulation*

In some units the recorded activity could not be correlated with any particular sensory input nor with an obvious muscular movement, but was initiated by a brief sensory input of some sort and then gradually died away. Two examples are shown in Figs. 7 and 8, both recorded from the same site.

(i) In the first example (Fig. 7) there was no recordable activity before stimulation, but a large burst of many different units was seen when the lateral skin of the distal arm was momentarily touched. There was a small behavioural response; the suckers nearby reached briefly upwards towards the stimulated area and then relaxed again. 'Spontaneous' sucker movement of this sort produced no activity in the recording site, so the observed activity was probably not proprioceptive, and stimulation produced no muscular contraction, suggesting that it was not motor either. The activity initiated by the stimulus persisted for some minutes, gradually dying away.

(ii) It was noticed that if the table on which the preparation lay was shaken there was often a big burst from a previously silent or near-silent site. To investigate this, the table was struck with a microswitch triggering the CRO. Fig. 8 shows that the consequent burst did not begin until 200–400 msec. after the stimulus, and that the resultant activity continued for some 7 sec. There is no evidence that the sense organs of the arm would perceive this stimulus, but the statocyst certainly would (Maturana & Sperling, 1963). The hypothesis that the recorded activity is initiated cerebrally as a result of statocyst information is supported by the long delay, which would be adequate for several synapses and transmission down some 15 cm. of nerve cord which separated the brain and the recording site.

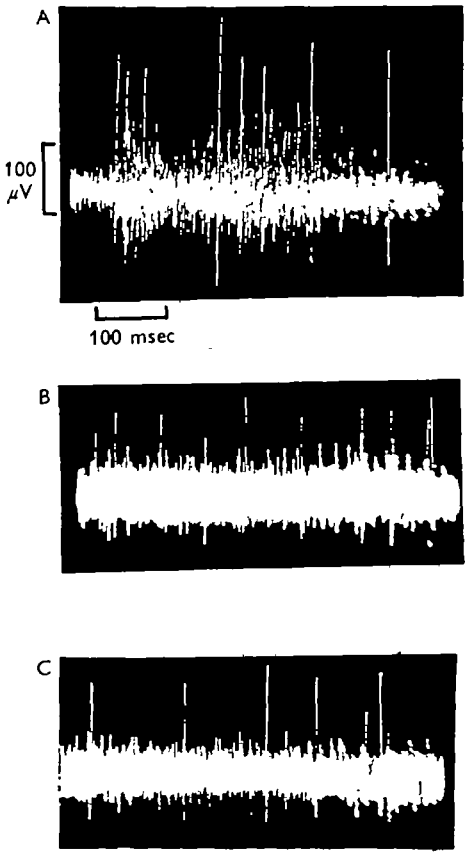

Fig. 7. Recordings from a site in the dorsolateral longitudinal tracts of the axial cord. A. Response to a momentary tactile stimulus applied to dorsolateral skin distal to the site of recording. B and C. Activity recorded from the same site after 2 and 4 min. respectively without further stimulation. There was no appreciable activity at the site before the stimulus. Further explanation in the text.

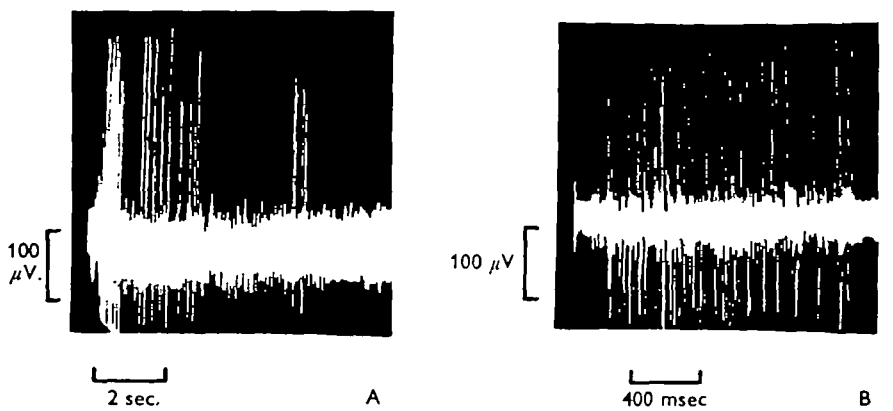

Fig. 8. Recordings from a site in the dorsolateral longitudinal tracts of the axial cord (same site as Fig. 7). The sweep is triggered at the moment that the table supporting the preparation is tapped; the effective stimulus appears to be vibration transmitted to the animal. A, slow sweep speed, B, faster sweep speed. Extensively retouched to show only the larger amplitude spikes. Further explanation in the text.

In both these examples the response habituated or tired rapidly. If the stimulus was repeated every few seconds, only the first elicited any activity, while the interval had to be several minutes before no waning was noticeable. Maintained activity might be due to an increased and maintained tonus in the arm muscles. However, electrical stimulation even with high currents gave no motor reponse, but only, after some time, an irregular protrusion of some distal suckers and searching movements, as if looking for a stimulating object. This suggests that the recorded units are connected with integrative sensory tracts.

Although these two examples were recorded at the same site and have similar general properties, it will be seen from Figs. 7 and 8 that their detail is quite different. The response to vibration lasted only a short time, less than 10 sec, while that to touch lasted several minutes. Further, in the former the various units all fire for more or less the whole burst, while in the latter there is a gradual dying away of the small fibres, until at the end there is only one large axon still recorded.

Observation at other sites suggest that this maintained activity after stimulation might be due to 'arousal' or dehabituation, for after the response to a tactile stimulus had waned it could sometimes be brought back transiently by shaking the bench or prodding the animal elsewhere, though these stimuli were not necessarily recorded in the same interneurone.

DISCUSSION

(1) *Sucker receptors*

Graziadei (1962) has shown that there are very numerous primary receptors on the sucker rims in *Octopus*, and has suggested (1964) that pressure is signalled by pear-shaped cells lying in the lower epithelium. The axons of the sucker sensilla run through a plexus formed by collaterals of their own axons and also those of the axons of sub-epithelial encapsulated nerve cells. There are synapses between the sensory axons and the subepithelial cells both in the plexus and on the some of the latter (Graziadei, 1965*b*). It is not known as yet whether sensilla of the different morphological types synapse on the same cell, nor what proportion, if any, send their axons without a synapse to the axial ganglia. Young (1965) inclines to the view that the majority run direct to the axial ganglia, but his estimate of sensory fibres entering the axial ganglion is at least four times smaller than Graziadei's estimate of the number of receptors on the sucker.

Whether the axon which is recorded so readily in the afferent nerve from the sucker is pre- or post-synaptic, there is no doubt that its effective sensory area is narrowly limited, certainly not more than 0·01 mm. across, and probably much less, and any lateral spread through the collaterals from outside this area is not enough to initiate action potentials. From the records obtained it seems to behave as a single fast-adapting mechanoreceptor of relatively low sensitivity but with no unusual features. Rossi and Graziadei (1958) report that the sucker nerves contain mainly small axons between 2 and 5 μ in diameter, but with a few up to 10–12 μ, and consider that these larger axons resemble in their structure the giant fibres of the mantle connectives. It may be that these are the tactile units described, and that their size accounts for the relative ease with which they recorded. However, these large fibres are said to ramify repeatedly peripherally, which argues against this identification.

No good records were obtained from any other type of receptor, but it is obvious from the anatomical descriptions and from the responsiveness of interneurones in the cord to a great variety of inputs that much more information is being transmitted in these nerves. Possibly if the nerve trunk were split down, other units would be apparent. EM cross-sections of the sucker nerves are now urgently required.

(2) *Proprioception and representation of muscle contraction*

Even allowing for the large size of the axial cord and the small size of a recording electrode, the complete failure to find information being transmitted about arm movement of position or muscle tension is in marked contrast to the situation in the neuropile of the axial ganglia, where at least some of these types were found readily. This picture fits well with what is known from other types of study of the organization of the cephalopod CNS. It can be shown behaviourally that there is a complex reflex system operating at the level of the single sucker and of adjacent groups of suckers which demands proprioceptive information, and lesion and stimulation techniques have shown that the nervous pathways involved are confined to the ventral surface of the cord linking succesive ganglia (ten Cate, 1928; Rowell, 1963). Interganglionic tracts of this sort are readily seen in horizontal histological sections of the cord. Wells (1961) has shown a stretch reflex in a loaded arm which must require at least local reflexes with proprioceptive inputs, and Graziadei (1965*a*) has described receptor cells which appear to fill this requirement. However, octopuses cannot learn discriminations based on proprioceptive inputs (Wells, 1963, 1964) and there is no evidence that proprioceptive information from the arm reaches the brain, though there is clearly transfer of positional information from one arm to another. Although it is impossible to demonstrate a negative proposition by a sampling technique, the present results support the view that most proprioceptive information is utilized only locally.

(3) *Tactile interneurones of the dorsolateral axial cord*

Perhaps the most striking thing about these interneurones is how common they are. They can be found almost anywhere on or in the cord. Simply moving the electrode slightly will show that it is recording from only a very small area, so it is unlikely that all records are of widely spreading potentials from a few large units. As even the small sample represented in a single recording shows considerable ability for spatial localization, it is clear that the animal has at a cerebral level the information to locate a tactile stimulus with great precision. Next, the tactile interneurones show great economy in their signalling. All that have been examined habituate rapidly, all are phasic, and many are 'novelty units' such as have been described from the vertebrate and insect visual system, responding only to something new in a large sensory field. An active unit is rarely found if changing stimulation is not being supplied. They appear to habituate to temporal patterns of stimulation as well as to maintained stimulation, and have the very interesting property of responding mainly to 'unexpected' inputs, as can be seen while recording from units which serve an area of arm which is being actively moved around. Contact with the tank in which it has been sitting for some time, or with its own body, or with a fixing nail, produces no response, but a new object will cause a burst of activity and investigation by the affected suckers.

Clearly, complex integrative processes have occurred before the level of these recordings, presumably at the synapses in the rim of the sucker (Graziadei, 1965*b*), and/or in the neuropile of the axial ganglia. It is not known how many synapses there are between the receptor and one of these cord interneurones, but the axial ganglion neuropile is known to be capable of organizing quite complicated behaviour patterns, and there are also the preganglionic synapses in the sensory system. In many respects the tactile interneurones of the arm are reminiscent of audio-visual interneurones in the brain of the locust (Horridge, Scholes, Shaw & Tunstall, 1965). These were thought (Horridge, 1965) to be third-order or higher interneurones. Some idea of the anatomical possibilities for integration in the axial ganglia can be derived from the estimates of numbers of nerve-cell nuclei and of axons given by Young (1963, 1965). The figures given for axon numbers, especially where they refer to mixed or sensory nerves, must be treated with caution, for they were made with the light microscope and comparison between light microscope and electron microscope estimates of axon numbers in invertebrate nerves have shown that the former method may underestimate by anything up to 10 times (Nunnemacher, Camougis & McAlear, 1961; Rowell, 1964). Young's estimates for the relevant parts are:

Cells in one axial ganglion about $1{\cdot}3 \times 10^5$

Sensory fibres in sucker nerves from one axial ganglion } about 3000.

Total motor axons from one axial ganglion } about 2000.

Afferent axons in axial cord about $1{\cdot}8 \times 10^4$

Efferent fibres in axial cord of which 2000 are chromatophore fibres } about 4300.

Making the assumptions that:

(*a*) all afferent axons of the cord have their somata in the axial ganglia, not in the brain, and thus average about 80 per ganglion;

(*b*) each motor axon arising from the axial ganglion corresponds to a different cell;

(*c*) a relatively small proportion of the motor axons leaving the axial ganglia are chromatophore fibres, which according to Young have their cell bodies in the brain;

(*d*) the axons which transmit sensory information to neighbouring ganglia along the inter-ganglionic tracts and which are probably interneurones arising in the ganglion are not more numerous than the original afferent sensory axons to the ganglion, that is, about 3000;

then, less than 10^4 of the ganglion cells can be allotted known functions, leaving more than 10^5 cells for intraganglionic neuropile. So far as is known, there is no memory store in the nervous system of the arm. Presumably the smaller fraction of this neuropile is concerned with decoding motor commands from higher motor centres (there is evidence that the motor system of *Octopus* is organized in a hierarchical manner (Wells, 1965)), which leaves the major part of these 10^5 cells to integrate sensory input. This figure is enormously large for an input of only 3000 axons; for comparison, the insect segmental ganglion has a sensory input of about the same size as the octopus

axial ganglion, but typically contains less than 10^3 interneurones. Even if the figure 3000 is 10 times too small, which is unlikely, and if also more of the ganglion cells are concerned with chromatophore function than Young allows (see Rowell, 1963), the ganglion still contains enough machinery for the most sophisticated computations.

The less rapidly adapting units which bring information centrifugally are presumably capable of co-ordinating the animal's ability to move the tip or distal part of the arm to a site of stimulation higher up the arm. It has been suggested (Rowell, 1963) that even an amputated arm may be able to do this, which would preclude cerebral control. These axons can perhaps be thought of as local 'help' lines, similar in function to those found running between axial ganglia, but serving a longer distance.

SUMMARY

1. A method for recording nervous activity from the nervous system of the arm of *Octopus* is given. Difficulties of mobility and vasoconstriction are reduced by brain lesions.

2. Three areas were recorded: afferent sucker nerves, axial ganglia, and the dorsolateral axial cord.

3. The sucker nerves include large tactile units corresponding to discrete parts of the sucker rim. These are fast-adapting, phasic, not very sensitive, and are located in the area of motor innervation of the same nerve.

4. Two types of interneurones were found in the axial ganglia, responding to either tactile stimulation of their own or neighbouring suckers, or to proprioceptive input from their own sucker. Motor units to the sucker musculature were also found.

5. Almost all recorded units in the dorsolateral axial cord were interneurones receiving tactile input. They have the following characteristics:

(*a*) they are rapidly adapting, often phasic, and show little or no 'spontaneous' activity.

(*b*) they habituate rapidly to even complex patterns of stimulation and discriminate between them, behaving as 'novelty units'.

(*c*) different sites of stimulation are discriminated by change in both the number of active units and their temporal patterning. The smallest area shown to be separately represented is the rim of one sucker.

(*d*) prolonged activity can be initiated by a brief initial stimulus, which is without apparent correlated motor output.

(*e*) stimulation of areas outside a unit's sensory field can lead to activity in that unit or to dehabituation in a previously active unit.

No proprioceptive representation was found.

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