

EXCITATORY AND INHIBITORY PATHWAYS IN THE ARM OF *OCTOPUS*

By C. H. FRASER ROWELL

*Department of Zoology, University of Cambridge**

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The octopus is an exceptionally strong, active and manipulative animal. The structure of the arms enables the animal to move over any sort of terrain, to apply a formidable grip or pull, to make most delicate movements, such as picking at tight knots in wet cotton until they loosen, to reach and clean any part of the body, or to make delicate sensory discriminations. The great learning ability of *Octopus* was first shown for visual problems by Young and Boycott and their school, but Wells and Wells have subsequently shown an equal ability to deal with tactile information from the arms (see Wells (1962) for reviews). The arms contain a large part of the central nervous tissue of the animal, with intrinsic sensory, interneural and motor elements, possess organized reflex systems, and are capable of quite a lot of autonomy. It is thus good material in which to study the lower organization of an invertebrate central nervous system.

Preliminary experiments on the capabilities of the arms, as distinct from the brain, were carried out by v. Uexküll (1894) and ten Cate (1928); apart from these studies and a large number of studies on chromatophores (reviewed by Parker (1948)), our knowledge is confined to anatomical descriptions. The following account is a further step towards physiological mapping.

ANATOMY

The nervous anatomy of the arm has been described by several authors, of whom the most recent and comprehensive are Rossi & Grazeidei (1954, 1956*a, b*, 1958). The main features are shown in Fig. 1. There is an axial nervous 'cord', the medulla, enclosed in a jelly-like material characteristic of the cephalopod C.N.S., and surrounding by a connective tissue sheath which also serves for muscle attachment. The medulla is made up of both cell bodies and axons, the former being peripheral. It is thickened opposite every sucker, which gives it a (spurious) segmented appearance, and from these areas, the 'axial ganglia', nerves run ventro-laterally to and from the sucker. Some of the sucker nerves run via the small subacetabular ganglia, one of which is located in the neck of each sucker, and which receives mainly sensory axons from the sucker rim. Sensory and motor nerves run radially between the medulla and all other parts of the arm. Some run via, and may indeed form, the four small longitudinal lateral or para-axial nerve 'cords', which run between the four blocks of mainly longitudinal muscle which surround the medulla.

The muscle systems were described in detail by Guérin (1908). Apart from the main longitudinal muscles, transverse fibres run vertically and horizontally across the

* Present address: Department of Zoology, Makerere University College, Kampala, Uganda

arms, and there are also three layers of oblique muscle fibres which run around the arm in different orientations; when all contract simultaneously the effect is as of a circular muscle system.

The suckers have a complex intrinsic musculature, including longitudinal, radial, meridional and circular (sphincter) muscles. A third system of muscles (the 'muscles extrinsèques' of Guérin) connect the sucker system with that of the arm and connect neighbouring suckers. The skin itself has an extensive dermal musculature, and the tissue between the skin and the outermost oblique muscle layer also contain a thin muscle sheet, figured but not named by Rossi & Grazeidei (1956*a*). This I shall refer to as the 'subdermal musculature'.

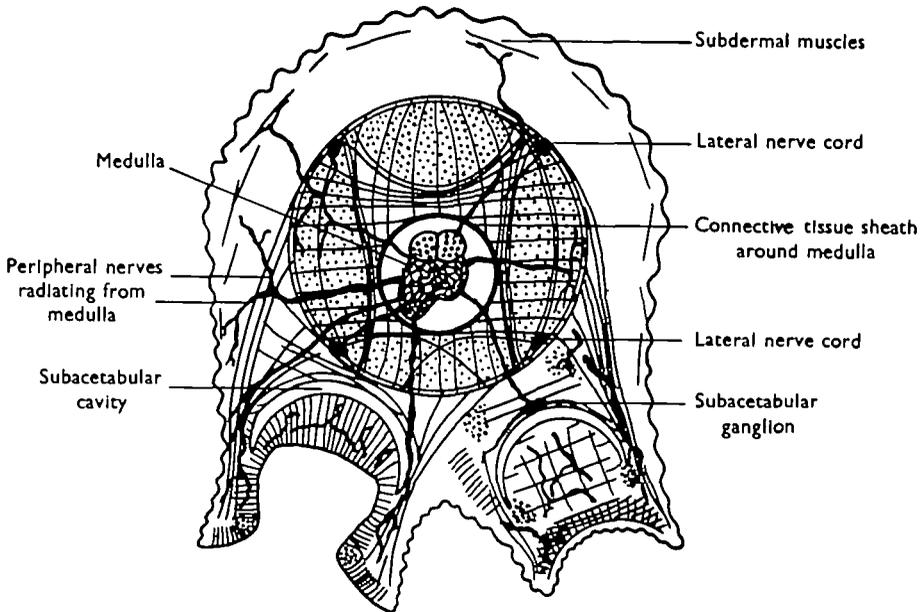


Fig. 1. Transverse section of the arm of *Octopus*, to show the main muscles and nerves. Simplified and diagrammatized after Rossi & Grazeidei (1956*a*). Muscles running in the plane of the paper are represented by lines, those running perpendicular to it by dots. The radiating peripheral nerves (thick lines) are more numerous than shown; those which are shown are stylized, and their fine details have no anatomical support.

METHODS

An arm was cut from an octopus (*Octopus vulgaris*), and the proximal portion was fixed to a block of wood with two nails, inserted so as to avoid the medulla. The wood was fixed in a bath of circulating aerated sea water at the same temperature as that in which the animal had been living immediately before the experiment. The distal part of the arm hung freely in the bath. Usually the arm was fixed lying on its side, so that at least one of the two rows of suckers was out of contact with the wood, and the medulla was exposed laterally over a length of about two or three suckers near the proximal end. The muscle was pinned back to allow access of electrodes. For some experiments the medulla was exposed ventrally or dorsally.

Nerves were stimulated with a 12 μ platinum wire, insulated to the tip in a glass

microelectrode; negative-going 1 msec. pulses were applied at 50 pulses/sec., and the sea water surrounding the preparation was earthed ('monopolar stimulation'). The pulse voltage was monitored on a C.R.O. Most of the nerves are set in connective tissue and all were stimulated under sea water. Thresholds measured under such conditions are meaningless, except relative to an immediately preceding trial in the same location, and will not be given.

There is a danger of confusion in describing chromatophore effects, as the expansion of the chromatophore is caused by contraction of its muscles. Throughout this account, the description will be in terms of the chromatophore, not its muscles, unless otherwise stated.

RESULTS

I. *Effects of amputation*

When the arm is amputated, the most dramatic effect is an immediate tonic grasping by the suckers of any surface which they contact. This grasping is stronger than that normally elicited from the intact animal. It gradually slackens, and after about 15 min. most of the suckers are relaxed once more. If prodded, however, a sucker will grip again, and if the object is moved about, the grasp continues. Only if it is left quiescent does a sucker relax, which suggests that adaptation takes place in the sensory input. As the preparation dies, this grasping reflex decreases, but can usually be obtained up to 3 hr. after amputation.

The colour of an octopus at rest varies enormously, but is generally darker than the palest coloration of which it is capable, when all the chromatophores are contracted. When it is taken from its tank to amputate an arm the animal goes dark brown. When the arm is amputated, its dark brown colour lightens to one typical of the resting animal, usually an orange brown on the ventral (walking) surface, and an olive green on the dorsal surface. Under the conditions described, this coloration lasts for about 30 min.; after this, the chromatophores contract, and the arm becomes a dull white all over. The same effect is seen if the medulla is cut in an otherwise intact animal; distally, the chromatophores slowly contract, and little or no colour is seen again while the arm lasts (it is usually eaten off in the first few days). In both cases, however, the chromatophores remain responsive to both direct and neural stimulation, and can be made to expand maximally in this way. While still expanded after amputation they can be caused to contract instantly by cutting the appropriate nerve from the medulla. The maintained expansion of the chromatophores seen after amputation is thus due to an autonomous activity originating in the amputated medulla, which ceases some time after the operation.

A prick with a needle to the skin of the ventral or dorsal surface will give three responses when the preparation is fresh: a local flinching of the skin, due to contraction of the dermal and subdermal muscle layers, a movement of the whole arm withdrawing it from the stimulus, and flexion of the arm and protrusion of the suckers in a way likely to cause them to come into contact with the stimulating object. These observations are the same as those of ten Cate (1928), who gives a more detailed account. A painful stimulus to the sucker itself, such as a needle prick or a small electric shock, causes immediate withdrawal, due to a general contraction of the longitudinal muscles of the sucker, and if the stimulus is continued, a general movement

of the arm away from it. Less extreme stimuli, such as prodding with a blunt object elicit the grasping reflex described, including an orientation component towards the stimulus. ten Cate (1928) describes an increased recruitment of neighbouring, especially distal, suckers, which also attempted to grasp the stimulating object; this rarely happened in my preparations.*

The reflexes gradually diminish as the preparation dies. The local skin response persists longest, probably due to its contact with oxygenated water.

II. Effects of lesions to, and electrical stimulation of, the arm

A general topographical chart of the effects produced by stimulation of the medulla and its nerves is given in Fig. 2.

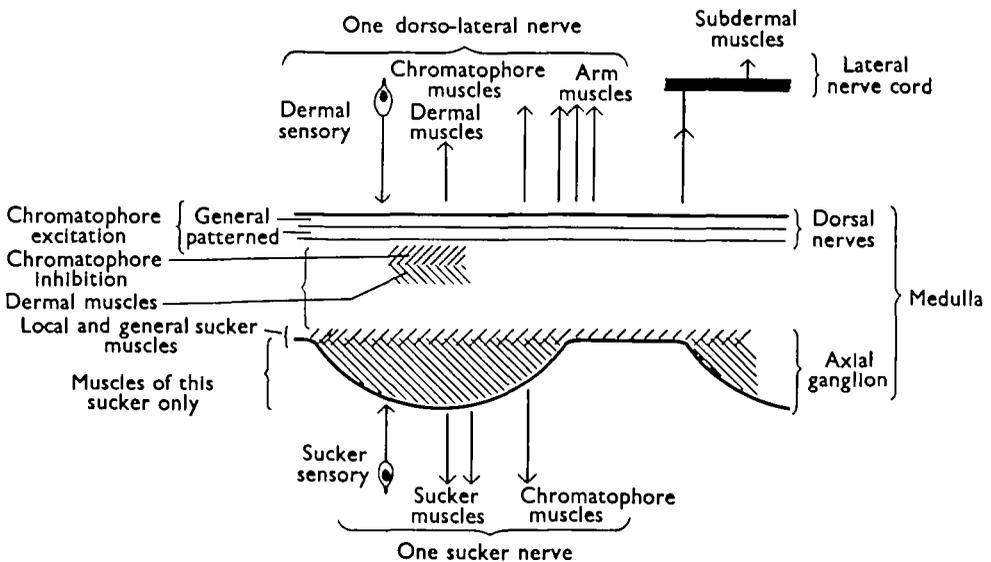


Fig. 2. A stimulator's guide to the brachial medulla of *Octopus*. A short length of medulla with one axial 'ganglion' is shown, and the effects obtained by electrical stimulation of different areas and of the nerves leaving it are indicated. Areas giving contraction of the dermal muscles and inhibition of the chromatophore muscles are small and poorly defined; the diagram shows only their dorso-ventral level on the medulla, and not their lateral extent, which is discontinuous. Unshaded areas control the general musculature of the arm.

A. The sucker

An electrical stimulus applied to a point on the rim of the sucker produces the following effects, in order of increasing threshold or latency or both.

- (1) Local withdrawal of the affected area of the rim (longitudinal sucker musculature).
- (2) Sudden withdrawal of the entire sucker (longitudinal sucker musculature).
- (3) Local chromatophore expansion at the point of stimulation.

* Slight discrepancies between the results of ten Cate and those presented in this paper are probably due to the different experimental conditions. It is to be expected that the intact, though blind, animal, at rest in its tank and gently stimulated via its own sense organs will give more complex responses than the amputated arm directly stimulated with electrical current. It is encouraging that the results are in fact so similar.

(4) Spread of definite limited pattern of chromatophore expansion to immediately proximal and/or distal areas. (Proximal or distal relative to the stimulating electrode.)

(5) Contraction of circular muscles of sucker rim.

(6) Spread of this effect (5) to adjacent ipsilateral distal suckers, occasionally 1st proximal.

(7) Contraction of ventral longitudinal muscles of the arm between the stimulated sucker and nearby proximal and distal suckers; this has the effect of bringing them closer to the site of stimulation. No protusion or grasping by them was seen (cf. ten Cate, 1928); indeed, the rim was usually contracted shut, due to effect (5) above.

v. Uexküll (1894) showed in addition that in *Eledone*, and thus presumably in *Octopus*, some conditions of electrical stimulation to a sucker would cause a proximal bending of distal suckers and a distal bending of proximal suckers, thus pointing them towards the stimulating object. This effect was not seen.

If the connexions between the sucker and medulla are all cut, then only effects (3) and (5) persist, i.e. local chromatophore expansion and contraction of the circular muscles of the sucker rim. A similar local chromatophore response can be produced on any isolated piece of skin; nervous transmission is not involved but only direct stimulation of the muscles. If stimulation of the neurally isolated sucker is further increased (voltage increase), local contraction of the sucker musculature takes place around the electrode; this too is presumably due to direct stimulation of the muscles.

As the nerves to the sucker are progressively cut where they leave the medullar sheath, the chromatophores of the area of sucker innervated by each nerve suddenly contract, giving a clear picture of the affected area. Each nerve also innervates the sucker muscles over the same area. If the sucker is gripping an object such as one's finger before denervation starts, the grip progressively slackens, and when between a third and half of the nerves are cut the sucker cannot maintain its grip.

Direct stimulation of the denervated portion of such a sucker produces only the responses listed for a completely denervated sucker, whereas the intact portion produces the normal range of responses. The denervated portion never produces contraction of an intact area (cf. effect (2) above); this shows that not only the motor signals to the sucker are transmitted from the medulla, but also that sensory input from the sucker, whether direct or via the subacetabular ganglion, travels in the same nerves. These are, therefore, mixed nerves. With one possible exception, no responses are obtained from stimulation of the sucker if the nerves coming from the medulla are cut. This implies that the subacetabular ganglion has no motor output to the sucker muscles. The possible exception is the contraction of the circular muscles of the sucker rim, which was noted as occurring after denervation. This could well be due to direct muscle stimulation, but ten Cate (1928) thought that it was abolished if the subacetabular ganglion was removed. I could not confirm this certainly, but if so it would show a short reflex path between sucker and rim musculature through this ganglion alone. Rossi & Grazeidei (1956*b*) found motor neurones in the subacetabular ganglion, but could not show certainly a connexion with the muscles; they thought that these neurones might control secretion.

Stimulation of the intact sucker rim produces a pattern of chromatophore expansion over nearby suckers (effect (4)). This expansion, being all-or-none and affecting only precisely limited areas, is clearly neurally controlled. It occurs most easily if the

electrode is pressed well into the skin, and the areas of coloration produced are usually similar for each sucker. It seems most probable that there are nerve cells (located in the medulla, not the brain, in view of reasons presented below) which send branches down the mixed sucker nerves, each innervating the chromatophores of several suckers, and that the observed effects are due to antidromic stimulation of such a nerve through its branch to the stimulated sucker. This explanation is supported by the fact that the pattern can affect proximal and/or distal suckers, presumably depending on which branch is stimulated.

If a sucker nerve is stimulated peripherally to a cut between sucker and medulla, local contraction of sucker muscles, especially longitudinal muscles, and local chromatophore expansion is produced. If the nerve is stimulated centrally to the cut, the response is complete as for direct stimulation of a normal sucker, except of course that the area denervated by the cut shows no response. These are the results expected from the deductions made above.

B. *Mechanisms controlling more than one sucker*

The account so far includes examples of effects spreading from one sucker to adjacent ones, as in the spread of contraction of sucker rims. This spreads slowly distally from one sucker to another, at high threshold, and appears to be due to a decremental spread, implying a multisynaptic path from sucker through medulla and thus to the next ipsi-lateral sucker. The occasional spread to only one sucker proximally suggests antidromic stimulation of a nerve which does not extend for more than two axial ganglia.* If the stimulating electrode is transferred to the ventral surface of the medulla, preferably between axial ganglia to avoid direct stimulation of sucker nerves, a range of effects influencing several suckers at a time is seen. These fall into two classes, as illustrated by the following examples:

(1) Retraction of the suckers, caused by contraction of all their longitudinal muscles. Affects both sides, but ipsi- more strongly than contra-lateral. Decremental conduction distally only for about 6–8 suckers, at rate of about 15 suckers/sec. Length of arm affected depends on intensity of stimulus. Implies a polysynaptic chain down both sides, with synaptic linkages between them. To this class also belong the spreading of circular muscle contraction, and the reflex of v. Uexküll, described above.

(2) Contraction of certain groups of sucker muscles, resulting in inward, outward, proximal or distal flexure, or protrusion, of all suckers of the arm, both distally and (with higher thresholds) proximally. Sites occur which affect both rows equally, or only one side. Action simultaneous down length of arm. Implies many through-fibres running the length of the arm, each synapsing with appropriate motor nerves of each sucker.

Other through-tracts in this area produce a grasping movement of all suckers which were previously relaxed and in contact with some object. This is a more elaborate movement than the above, and involves most of the sucker musculature. The synaptic arrangements between through-fibre and sucker nerves must be correspondingly complex.

* This account frequently mentions effects which spread distally but not proximally. I believe that the majority of these represent real differences in the physiological organization, but it must be remembered that the proximal regions are nearer to the site of amputation. This may influence the results,

The main results described in sections A and B, i.e. the sensory and motor pathways directly influencing the sucker, are summarized diagrammatically in Fig. 3.

C. The chromatophores

The local control of chromatophores is by fibres running in nerves radiating from the medulla, as already shown for the suckers (section A). Stimulation of any of these produces a small discrete area of expansion. The neuromuscular mechanism adapts very rapidly, and a given voltage will usually produce only a transitory expansion.

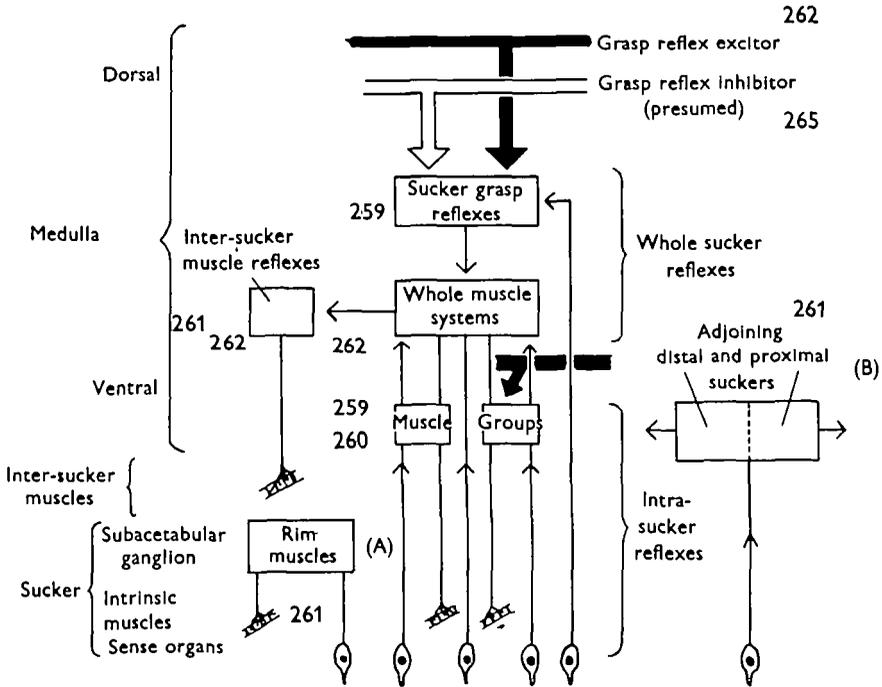


Fig. 3. Diagram of the nervous organization in the medulla affecting the muscles of one sucker of the arm. Anatomical levels are shown on the left-hand side. The numbers refer to pages in the text where the evidence for any detail is presented or discussed. The two reflex mechanisms labelled (A) and (B) are respectively those described by ten Cate (1927) and v. Uexküll (1894). The label 'whole muscle systems' indicates for example all the longitudinal muscles of the sucker, while 'muscle groups' would mean a small patch of the longitudinal muscles.

It is possible to stimulate one of these branches antidromically as described in section A, and thus show up the other areas innervated by the same nerve cell. Typically these consist of four to five patches on the dorsal and lateral surfaces of the arm, and some patches on three or four suckers. Usually they are similar areas for each sucker of the arm. Frequently different nerve branches control overlapping areas of chromatophores, but it has not been possible to decide whether different chromatophores are involved in these cases or not.

All these nerves are normally excited by the medullar output to the chromatophores shown in section I.

Stimulation of the dorso-lateral medulla excites through-tracts which activate

numbers of these subsystems, both proximally and distally, producing an over-all pattern which is characteristic of any one site. Increase of voltage apparently brings in more fibres of the same sort, and causes more of the surface to be affected. Yet more dorsally are tracts which cause all the chromatophores of the arm to expand to their maximum simultaneously, turning it a uniform dark brown. These last are apparently the fibres found by v. Uexküll (1894).

Ventral to these excitatory tracts and overlapping areas which produce movements of the arm musculature (section D) are small areas, relatively difficult to find, which cause a contraction of all the chromatophores over large areas of the arm. This effect can only be found in the fresh preparation, for thereafter the output to the chromatophores ceases anyway, and the chromatophores are at all times contracted. There is,

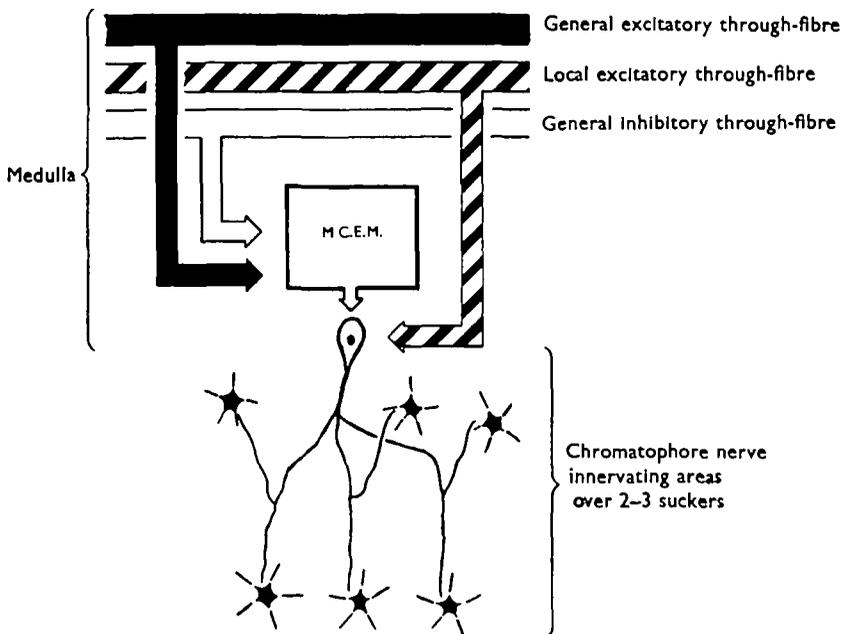


Fig. 4. Diagram of a possible nervous organization within the medulla controlling chromatophore expansion. The sites of action of the inhibitory and excitatory through-fibres are not certain; those shown are based on the assumption that the chromatophores are not doubly innervated. For other possibilities see the Discussion. M.C.E.M. = medullar chromatophore exciting mechanism.

relative to the excitatory tracts, a considerable latency of about 1 sec. with normal stimulating voltages. Usually the inhibition is more or less general, but some patterned inhibition was sometimes seen. The excitatory tracts, the chromatophore nerves, and the chromatophores themselves remained excitable during inhibition, as could be shown by using two electrodes simultaneously, and as far as could be seen, with the present rough method, the excitatory threshold was not appreciably raised by the inhibitory signal. This suggests that the inhibition may act on the normal motor output to the chromatophores (section I), rather than directly by an inhibitory output to the chromatophore muscles.

These results are summarized in Fig. 4.

D. *The arm musculature*

The main longitudinal muscles of the arm, which result in its flexion and contraction, are arranged in four blocks around the medulla. Between these blocks run the four lateral nerve 'cords', which might be thought to be associated with these muscles. Electrical stimulation of these lateral cords, however, produces no response from the longitudinal muscles (see section E below).

Stimulation of the medulla, especially in a dorso-medial region, produces vigorous flexion and waving of the whole arm. The effective area is not easily defined, which probably means that the responsible fibres run in the interior of the medulla. At threshold it is sometimes possible to produce a slow build-up of flexion and arm movements which gradually affect more and more of the arm distal to the stimulus. This suggests a multisynaptic transmission down the medulla.

Stimulation distal to a cut of only one of the many radiating lateral and dorsal nerves produces effects on local areas of skin and chromatophores only. A local flexion due to contraction of one segment of muscle never occurs, though it is clear that the motor output must leave the medulla via some of these nerves. When, however, a group of perhaps 10 are stimulated simultaneously with a large wire electrode, a local flexion is produced. This may be a meaningful result, or it may be due merely to electronic spread to the medulla.

In the same general area of the medulla are found sites producing local or general longitudinal contraction (of all four blocks of muscle) of the arm, or extension of the arm, caused by contraction of the oblique muscles of the arm; this last was often accompanied by a protrusion of all suckers (see section B).

E. *The dermal and subdermal musculature*

Local contraction of the dermal muscles is a common effect of medullar stimulation, and their motor output is transmitted through about half of the radial nerves. Local contraction of the subdermal layer, resulting in a wrinkling of the skin, was obtained occasionally from the medulla. It was obtained repeatedly from the lateral nerve 'cords'; Dr P. Grazeidei kindly dissected these out in a large specimen, and no other response was obtained from them.

The intact animal has great control over its skin texture, and can, for example, cause a striking papillose texture, where the head, body, and upper arms are decorated by regular papillae up to 5 mm. in length; the pattern is constant from individual to individual. This must demand precise control over dermal and subdermal muscles, and Boycott (1961) has obtained this effect by electrical stimulation of the anterior chromatophore lobe of the suboesophageal ganglion of *Sepia*. Presumably the nerves responsible must be present in the medulla or lateral cords. They were not identified.

DISCUSSION

(1) *Inhibition of sucker-grasp reflexes*

It is clear from the behaviour of the animal, and from the effects of amputation, that the brain must transmit inhibitory signals to the sucker-grasp mechanism located in the medulla (section I), as otherwise the animal would not be able to move around, much

less perform more complicated movements of the arms, such as handling objects. Behaviour also suggests that this inhibitory control is detailed and spatially labelled at least down to the level of a few suckers, for the intact animal is quite capable of letting go of one surface while retaining its hold on another. There are lesions to parts of the brain, e.g. the cerebro-brachial tracts, which have the effect of interfering with the sucker-release system (v. Uexküll (1895), B. B. Boycott (unpublished)), and which would fit in with these deductions. Such inhibitory tracts have not been found by direct stimulation of the medulla. It is expected that further work will reveal them; the present failure is probably due to a higher threshold or longer latency of the inhibitory system compared to the excitatory.

(2) *Inhibition and excitation of chromatophores*

It was shown in section I that chromatophore expansion was dependent upon an intact nervous connexion with the medulla, and further that the medulla of an amputated arm continued to supply the necessary output to the chromatophores for a considerable time. Sereni & Young (1932) showed that chromatophore fibres run direct from the brain through the stellate ganglion and on to the mantle, and Boycott (1961) has suggested that this direct cerebral innervation might be the rule for all chromatophores. It is known that the arm chromatophores are under nervous control of the brain, for changes in them can be caused by direct stimulation of the brain (Boycott, 1961), and the co-ordination of their colour change with that of the rest of the body is lost if the brachial medulla is cut. The present experimental evidence is interpreted as showing that the chromatophore-exciting mechanism is medullar, not cerebral, as spontaneous activity would not reliably continue for more than 30 min. in an isolated centrifugal axon. Tauc (1960) has shown that in the special case of the giant cell of *Aplysia* the spontaneous initiation of action potentials takes place not in the soma of the cell body, but in the axon between 0.35 and 2.0 mm. from the soma. This, however, is a very different thing from spike initiation up to at least 40 cm. from the cell body, as would be necessary in the present case; further, it would have to be postulated that the axon was capable of initiating the steady discharge at any point along its length, for chromatophore expansion is maintained regardless of where the amputation is made. The remaining possibility, that 'wound stimulation' causes continuous discharge in the amputated centrifugal axon, is dismissed, as (a) this does not happen with any of the other medullar systems investigated, and (b) Boycott's hypothesis would require a difference in this respect between section of the axon in the medulla and of the same axon in the nerve from the axial ganglion.

If the above interpretation is correct, it follows that there must be synapses between the brain and the chromatophores, at least (a) between the through-fibres, both excitatory and inhibitory, from the brain and the lower parts of the chromatophore system, and (b), in all probability, between the medullar chromatophore-exciting mechanism and the chromatophore nerves. It is of course possible that the cell bodies of the chromatophore nerves are themselves spontaneously active, and that they are the medullar exciting mechanism.

The existence of inhibitory fibres and of general and local excitatory fibres all affecting chromatophores of the same region suggests that the synaptic connexions within the medulla may be complex. The local excitatory fibres which control a

specific pattern over all the arm are of particular interest, for they simplify one's picture of the function of the chromatophore lobe of the brain (Boycott, 1953, 1961). While it was thought to contain the cell bodies of all nerves directly innervating the chromatophores, the problems of control and transmission were much greater.

The mode and site of action of the inhibitory and excitatory fibres remains to be discovered. They are through-fibres of central origin, as would be expected from, for example, the animal's 'dymantic response', which involves rapid chromatophore contraction over most of the body surface. Two of the most probable sites of inhibition are:

(1) The medullar chromatophore-exciting mechanism (see section I). In this case the result of inhibition would be to turn off the normal output to the chromatophore nerves. There may be support for this in that, during an inhibitory signal, the exciting mechanism remains usable at all levels (section II *c*), and further, in that patterns produced by inhibition are less detailed and defined than those produced by excitation.

(2) The chromatophore muscles themselves. This is not a new idea. It was suggested, for example, by Bozler (1931) and it is supported by Bullock & Horridge (1963) in their new review of the literature. The evidence on which their assessment is founded repays examination. Basically, the argument for inhibition acting on the chromatophore muscles rests on the belief that the normal degree of expansion of the chromatophores is due to an 'autotonus' of the muscles, independent of nervous supply. To quote from Bullock & Horridge's review: 'Stimulation of peripheral nerves when there is tonus in the chromatophores has often been observed to produce bleaching. This must mean inhibitory nerve fibres because the tonus does not appear to depend on steady outflow of motor impulses and hence there is nothing to cut off in the nerve.' This is directly contrary to the observation reported above, that section of a nerve from the axial cord to the skin causes an instant contraction of chromatophores over the area innervated. One suspects that the work on which this assessment is based was done on preparations which were dying, or at least in a very unnatural state. As stated above, the amputated arm retains its normal coloration for up to half an hour (though only in favourable circumstances; if kept out of water or too hot it goes white much sooner). Thereafter the chromatophores contract and the skin goes white. If it is kept still longer, the chromatophores expand again ('autotonus') and the skin becomes dark once more. Curious random colour changes, the 'wandering clouds', also occur. These changes are probably morbid. It is likely that the first loss of the normal colouration, which means the death of the mechanism that initially maintains it, signifies the end of the normal working conditions of the chromatophore. Bullock & Horridge (1963) quote two particular pieces of work to support the idea of normal autotonus and thus muscular inhibition. One of these (Bozler, 1931) used isolated pieces of skin from the belly of *Loligo*. In *Octopus* isolated pieces of skin reach the 'wandering clouds' stage almost at once, and if the same is true of *Loligo*, this is not serious evidence of *normally occurring* autotonus. The second is a report that 'section of the mantle connective causes an immediate paling whereas removal of the stellate ganglion causes an initial darkening, passing into blanching after some hours and slowly turning dark after a few days, presumably due to autotonus' (source and animal not stated). The result of section of the mantle connective is the same in *Octopus*, but section of the nerves running from the stellate ganglion to the mantle—

which is presumably the same in effect as removing the ganglion—also causes an instant paling, each nerve serving a distinct area of skin and mantle musculature. This area does not darken in the living animal, at least within 24 hr.

The discrepancies noted above may of course be due to differences between the chromatophores of different genera, or different parts of the body of the same animal. However, it seems clear that there is no pre-existing evidence in favour of an inhibitory supply to the chromatophore muscles of the arms of *Octopus*. The best that can be said for the histological evidence for such a supply is that 'older descriptions can only be said to be compatible but not to predict it' (Bullock & Horridge, 1963). Rossi & Grazeidei (1958, and personal communication) have shown paired motor nerves in many parts of cephalopods, but have not yet shown that this corresponds to a double innervation of any motor structure. Wilson (1960) has shown physiologically that there is slow and fast innervation of cephalopod muscle, with differing latencies, which would perhaps fit with the different diameters of the nerve fibres observed histologically, but he found no evidence of an inhibitory supply.

The present evidence seems therefore to favour the alternative first put forward, that the inhibitory control of the chromatophores acts within the axial cord on the nervous mechanism, and not peripherally at the muscles. Recordings from the nerves running from the axial cord to the skin during stimulation of inhibitory and excitatory areas would help to resolve this problem.

(3) *Reflexes*

The effects on chromatophores reported in these experiments have all been produced by electrical stimulation of nervous structures or chromatophore muscles direct. There appear to be no local reflexes involving normal sensory input and chromatophores; all control of them is cerebral.

The reflex grasping of the suckers, by contrast, appears to be entirely autonomous to each sucker and its axial ganglion. This very important mechanism—the animal secures its food in this way—is found to be modified centrally by both an excitatory input and presumably (see above) by an inhibitory one.

The arm in general has effective and complex evasive responses to painful stimuli, in which dermal, subdermal, arm and if necessary sucker, musculature are all involved. There is also a strong impression that when fresh the free portion of the arm is accurately moved to the area where an experimental lesion is being made; if this is so, it implies a considerable complexity of spatial representation in both the sensory input and motor output, and probably also muscle proprioception, which, with one exception, has not so far been described from these animals. Alexandrowicz (1960) has recently shown a muscle receptor organ from the mantle musculature of *Eledone cirrhosa*. He emphasizes, however, that its structure is such as to make deliberate histological discovery of further similar organs almost impossible in the absence of a foolproof method of staining nerve cells; the muscular components of the organ do not differ visibly from normal muscle fibres. Muscle receptors may indeed be common and widespread in cephalopods; under these circumstances negative evidence is not reliable. Proof of their existence in other parts of the body must therefore be looked for in the animals' behaviour and in reflexes such as those described. Unfortunately, the full evasive responses are short-lived in this preparation, and have not therefore

been studied. v. Uexküll (1894) notes that the muscular response of *Eledone*, with the exception of chromatophore effects, last much longer than those of *Octopus*; this animal might therefore provide an opportunity for studying these more complex features.

SUMMARY

1. The nervous system of isolated arms of *Octopus vulgaris* has been investigated with fine stimulating electrodes and lesions. The reflexes of the intact arm are in accordance with earlier reports.

2. The reflexes are abolished if the nerves from the medulla are cut. These nerves carry both sensory and motor signals, serving discrete areas of muscle and skin. The central mechanisms producing single-sucker reflexes are confined to the ventral 'axial ganglia'.

3. Stimulation in the ventral medulla influences many suckers simultaneously or progressively, implying both through-pathways and polysynaptic pathways.

4. The nervous supply to the main longitudinal and oblique musculature remains obscure. Motor effects are produced by stimulating the median and central medulla, and require intact peripheral supply. The lateral nerve cords do not affect the main musculature.

5. The dermal musculature is affected by stimulation in the medulla and of its peripheral nerves. Only the subdermal muscle layer is excited by stimulation of the lateral nerve cords.

6. Normal expansion of the chromatophores depends on an intact nervous supply from the medulla, and is not due to autotonus of the chromatophore muscles. The final chromatophore nerves are probably medullar, not cerebral, and each controls only a few patches of skin over short lengths of the arm.

7. Through-pathways, affecting many or all of these chromatophore nerves, run in the dorsal medulla. They include local and general excitatory, general inhibitory and possibly local inhibitory channels. Their significance, and that of excitatory and inhibitory supplies to the sucker reflex mechanisms, is discussed.

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