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A NEUROETHOLOGICAL INVESTIGATION OF THE PAIRED TENTACLES OF *XENOPUS* TADPOLES.

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July, 1978.

A report submitted to the Department of Zoology, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the Degree of Master of Science.

PREFACE.

This work incorporates the results of research undertaken in the Department of Zoology, University of the Witwatersrand, Johannesburg.

The results of the present study are the original work of the author and have not previously been submitted in any form at any university.

Kelly

Patrick John Kelly

ACKNOWLEDGEMENTS.

I wish to sincerely thank Dr. A.J. Cannone for advice, guidance and encouragement during his stimulating supervision of this research.

I thank Mr. J.V. Thompson for photographic assistance and for printing all the photographs used in this work. I also thank Antoinette for her much needed assistance in the typing of this manuscript.

This work was supported by a Senior Bursary from the University of the Witwatersrand, Johannesburg.

ABSTRACT.

A neuroethological investigation of the paired tentacles of Xenopus tadpoles was undertaken. The tentacles were found to be extremely sensitive mechanoreceptor appendages. They respond to changing tactile stimuli and changing states of bending, but not to constant tactile stimuli or constant bending states.

Using this physiological data behavioural investigations were undertaken to establish the functional roles of the tentacles. The importance of the tentacles to the tadpoles was shown by the tentacle withdrawal reflex which was described.

Previous hypotheses of the tentacles' function were disproved and new hypotheses, derived from the physiological findings, were tested.

From morphological considerations, an anterior 'blind spot' was hypothesised. This, coupled with recordings of optical activity, suggested that the most probable functional role of the tentacles is to probe the anterior environment. In this way the tentacles compensate for the inherent lack of manoeuverability of the tadpoles, especially in preventing their becoming trapped in vegetation.

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GENERAL INTRODUCTION.

Xenopus laevis is an aquatic animal. The adults are most commonly found settled on the bottom of stagnant ponds and only rarely in running water (Deuchar, 1975). They breathe air and can often be observed to surface and gulp air in ponds where they are present.

Breeding occurs over a period of three to five months during the spring in temperate regions (Deuchar, 1975). During copulation the male remains in a position of amplexus while the female releases the eggs. Between 500 and 1000 eggs are released during one spawning. These eggs are released in ponds of water away from strong currents (Brown, 1970). The eggs are independent of one another, unlike in other frogs where the eggs are in a continuous jelly mass.

The tadpoles hatch within three days of spawning, at stage 38 of development (all stages referred to are according to the classification of Nieuwkoop and Faber, 1967). When the tadpoles hatch they have two external gills, and a cement gland situated just ventrally to where mouth perforation will occur (Nieuwkoop and Faber, 1967).

The tadpoles hatch out head first through the vitelline membrane and then attach to the water surface or any firm object in the water by means of a mucus thread secreted from the cement gland (Roberts and Blight, 1975). While thus attached the animal remains motionless. If the animal is 'provoked', it will swim off until its cement gland contacts something firm in the water, even the surface film, when it reattaches and swimming ceases (Roberts and Blight, 1975). During this 'cement gland' stage the tadpoles do not feed but are still absorbing yolk present in the gut.

The cement gland stage ends at stage 45 (Brown, 1970) when the mouth perforation is complete and filter feeding starts.

While the adults are carnivorous, feeding on most forms of living or dead organic material, the tadpoles are filter feeders. Water is gulped in via the mouth and then passed over the gills of the animal which are now internal. Food material and other micro-organisms are trapped in mucus present on folds in the pharynx (Wager, 1965). This mucus is continually flowing into the gut by means of ciliary action (Wager, 1965).

Just before mouth perforation is complete, at stages 43 and 44, the tentacles can be observed as small bumps on either side of the mouth slit which begins to perforate through at stage 40 (Nieuwkoop and Faber, 1967). The short tentacles of the animal can be observed at stage 46 (Nieuwkoop and Faber, 1967). These tentacles grow anteriorly and reach a maximum length, up to half the body length, by stage 52.

Following mouth perforation and the beginning of filter feeding the tadpoles adopt their characteristic free-swimming position in the water. This is a head down position with the tail pointing upwards at an angle of 45 degrees to the horizontal (Weisz, 1945). This free-swimming position is due to a delicate balance between the downward force exerted by the flickering tail tip and the buoyancy of the inflated lungs (Gradwell, 1971). Swimming depth is controlled by the flicker frequency of the tail tip (Gradwell, 1971).

The animals are not observed to move around much in the ponds where they live. Position changes do however occur following startling of the tadpoles. This results in the tadpoles quickly flipping their entire tail from side to side which causes the animals to move off in a downward direction at a gradual angle. This startle response seldom results in the tadpoles moving much more than about twenty centimeters. The tadpoles never appear to move towards vegetation cover or to actually descend and remain on the bottom (Van Dijk, 1972). This escape behaviour, where the tadpole does not swim down to the bottom, is probably associated with their transparency and reflectivity which causes a predator

to observe the tadpole's shadow on the bottom more easily than the tadpole itself(Van Dijk, 1972).

In nature Xenopus laevis tadpoles can mostly be observed in groups, the individuals of which all seem to have a common orientation (Weisz, 1945). Van Dijk (1972) points out that this is not necessarily gregariousness but may be a form of rheotaxis. The individuals of the group are not always at the same stage of development.

During the free-swimming period, the tadpoles can also be observed to make frequent rapid swims vertically up to the surface whereupon they immediately turn around and swim back down vertically again, 'spitting' out a bubble of air on the way down. This is probably a sign of the lungs of the tadpoles being functional at a fairly early age (Brown, 1970). The air-filled lungs of the tadpole probably serve to aid the buoyancy of the tadpole in its free-swimming position (Gradwell, 1971).

During the free-swimming period the limbs of the tadpoles develop - the hind limbs becoming the first to be functional at stage 58/59. When functional they aid the tadpole primarily in the startle response and only apparently slightly in maintaining the free-swimming position. The tail still remains the prime means of maintaining the free-swimming position. The forelimbs, when functional, do not appear to aid locomotion at all (Brown, 1970).

The tentacles begin to be reabsorbed at stage 59 and accompanying this process they begin to shrivel up (Nieuwkoop and Faber, 1967) and the tentacle base shifts to a more lateral position on the head. Tentacle reabsorbtion is complete by stage 61 (Nieuwkoop and Faber, 1967). The tentacles are very frail appendages and are often broken off at various lengths before reabsorbtion begins. This is especially the case where tadpoles are kept in shallow trays in which they can fairly frequently be seen to swim forcefully into the sides.

During and after tentacle reabsorbtion, Brown (1970) has noted that the eyes of the tadpole shift from being laterally placed on the head to a more dorsal position, "enabling the animal to see in front of it."

The tail also begins to become reabsorbed (stage 62) at the end of tentacle reabsorbtion, and although as a result it becomes shortened, it still serves to maintain the tadpole in its characteristic free-swimming position. Once the tail is present only as a short stump (stage 64), and metamorphosis is almost complete, the tadpole sinks to the bottom and remains there, surfacing periodically for air. From this stage onwards the tail is non-functional.

After metamorphosis, the froglets are much smaller than adults, but following an eighteen month growth period, the froglets attain adulthood and can start breeding (Deuchar, 1975).

The purpose of this investigation was to try and ascertain why Xenopus tadpoles have tentacles during particular stages of their development and not in subsequent or previous periods.

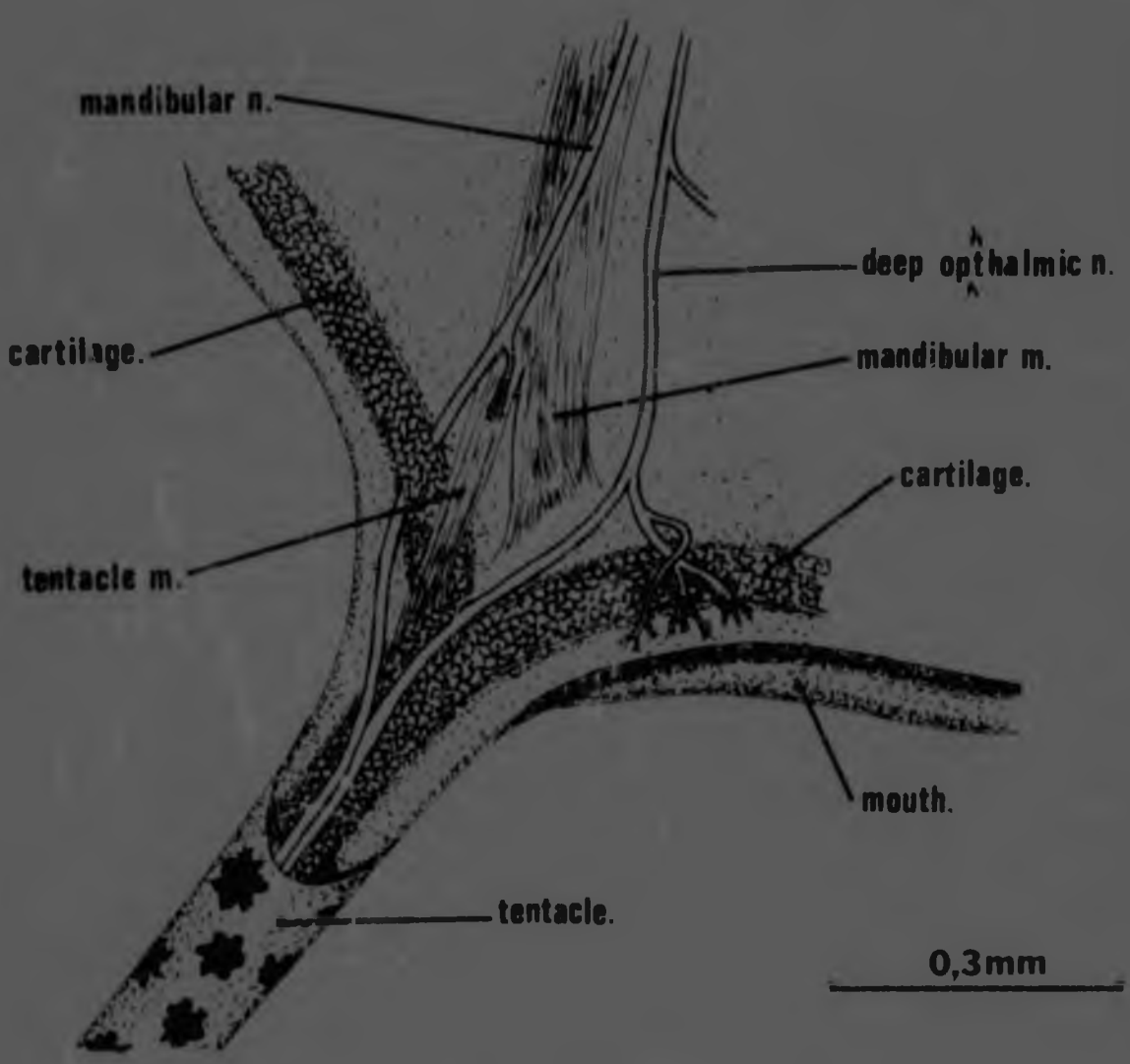

Figure I. Diagram of the relevant morphology at the base of the tentacle of Xenopus laevis. Dorsal view with skin removed

THE SENSORY PHYSIOLOGY OF THE PAIRED TENTACLES OF XENOPUS TADPOLES.

I. I Introduction.

The gaining of an understanding of the possible functional role or roles of the tentacles will be greatly facilitated if it is known which particular sensory stimuli are detected by the tentacles. Since the tentacles are only present during the larval stages it is probable that all the sensory input from the tentacles is relevant to the animal. Intuitively it would seem unlikely that certain sensory inputs are present which are not effective due to lack of neural organisation in the still developing brain.

Fig. I illustrates the gross morphology of the tentacle, its innervation and its musculature. Two sensory nerve bundles innervate the tentacle. These are the deep ophthalmic and mandibular nerves. A cartilage rod extends along the length of the tentacle. A branch of the mandibular muscle inserts onto the base of the cartilage rod (Paterson, 1939; Ovalle, 1976).

Fabian, Hanrahan, Marks and Coombe (personal communication) found that both myelinated and unmyelinated neurones are present centrally within the tentacle. Ovalle (1976) found that most of the sensory neurones within the tentacle terminate within the epidermis, either in "close association with ordinary epidermal cells" or, as the majority do, in intimate synaptic contact with granulated Merkel cells. Ovalle (1976) states that such Merkel cell/neurite complexes are profusely scattered over the surface of the tentacles. This is in contrast to the findings of Nafstad and Baker (1973) that Merkel cells comprise about 0,3% of the total number of epidermal cells in Rana Pipiens skin.

Merkel (from Munger, 1965) originally found what he termed 'Tastzellen' and what are now commonly called Merkel cells. He considered these to be cellular transducers of physical stimuli to the neurite, that is mechanoreceptors. Similarly Nafstad and

Baker (1973) have suggested that Merkel cells and the nerve fibers synapsing with them may constitute a tactile apparatus in the skin of Rana pipiens. However, Whitear (1974) maintains that there is no real evidence for Merkel cells having a receptor function. Instead she maintains that they may be "part of an efferent communication system between nerves and the skin or end organ."

Munger (1965) suggests that since neurites seldom end on Merkel cells in the oppossum snout, but rather ascend into the epidermis after coming into apposition with the Merkel cell, it is possible that Merkel cells modulate growth or function of the neurite.

Iggo and Muir (1969) found that in cat skin, touch corpuscles or domes are present which contain tactile cells (Merkel cells) at the base of the epidermis. Each touch cell or Merkel cell was found to be innervated by one large myelinated axon, although one of these axons can innervate up to five corpuscles, but two is the norm. These sense organs can be identified in many species, including man (Iggo and Muir, 1969).

They found that no response could be monitored from the touch corpuscles in the absence of intentional stimulation, but when a smooth probe is traversed across the corpuscle a brief high frequency burst — quoted by Iggo and Muir (1969) as greater than 1000 impulses/sec — of impulses can be recorded from an afferent unit in the nerve bundle. They also found that a static response can be monitored if the displacement of the mechanical stimulus is sufficient. This static response comprised two phases - a phase of rapid adaptation and then a phase of slow adaptation which usually lasts for longer than ten minutes.

Although not specifed by Ovalle (1976) it appears that the Merkel cells of the tentacle are generally scattered in the skin as opposed to being confined to specialised sensory regions of the skin.

Thus, since many of the physiological characteristics of the

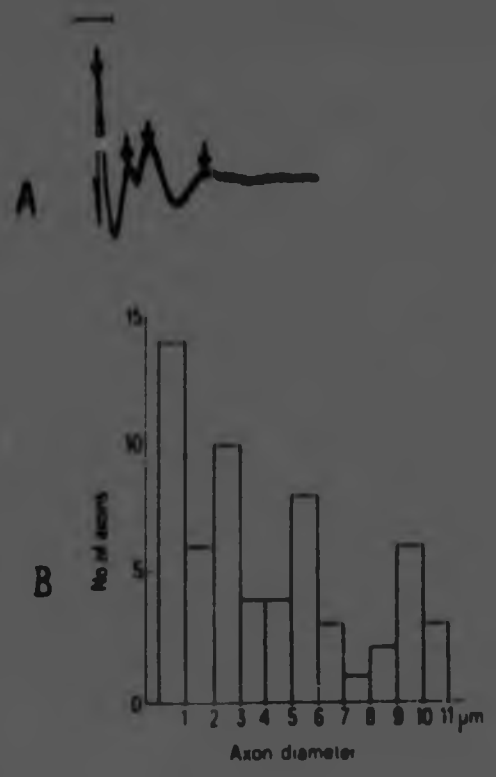

Figure 2. A. Distribution of conduction velocities of dorsal cutaneous afferents in Rana pipiens. Four peaks are discernable in nerve recordings from supramaximal stimulation of a distal portion of the nerve. Calibration: 2msecs.

B. Size distribution of neurones in the dorsal cutaneous nerve in Rana pipiens.

(Both reproductions are from Spray and Chronister, 1974)

touch domes may be dependent on the structure of the corpuscle (Iggo and Muir, I969), possibly different responses should be expected from the Merkel cells in the skin of the tentacle. As yet no work appears to have been done on the neurophysiological response of Merkel cells in amphibian skins, or on their possible contribution to the behaviour of the animal.

Catton (I958), working on portions of the calf skin of Rana temporia, described four categories of mechanoreceptor. Spray and Chronister (I974) working on cutaneous afferents in Rana pipiens have confirmed that there is a separation of receptors and their axons into four different populations. Each population type has a different receptor type, conduction velocity and axon diameter (Spray, I976).

When recording from the whole nerve following supramaximal stimulation, Spray and Chronister (I974) found four peaks of compound action potentials. These peaks occur as a result of compound action currents from axons of similar diameter. The latency of the peaks is related to axon diameters (Fig. 2).

Catton (I958) tentatively assigned different sensory functions to the populations by analogy with comparable data from mammalian and human experiments and taking into account the effective stimulus mode for each population.

The four sensory functions were designated as follows:-

Fiber type.	Function.	Conduction velocity.
a	Fast touch.	20 - 30 m/sec.
b	Slow touch.	I0 - I5 "
c	Vibration.	5 - I0 "
d	Pain.	0,I - 0,3 "

Similarly Spray (1976) has concluded that there are four distinct fiber populations which correspond to four distinct sensory modalities. He agrees with the functions attributed to the

'a', 'b' and 'd' fiber populations by Catton (1958) but maintains that the 'c' fiber type may in fact respond to cold. Catton (1958) states that the possibility exists, on the basis of the findings of Hensel and Zotterman (1951) that mechanoreceptor responses can be evoked by thermal stimulation, viz., that the 'c' fibers may additionally respond to chemical or thermal stimulation.

Spray (1976) states that the 'a' fiber population has free nerve endings in the epidermis. Neurones of the 'b' fiber population terminate as superficial encapsulated endings and neurones of the 'c' and 'd' fiber populations terminate as dermal free nerve endings.

As yet no investigation into the developmental aspects of these four fiber populations has been undertaken. Similarly no work appears to have been done on mechanoreception in adult Xenopus skin or in the late tadpole skin. However, Roberts and his various coworkers (Roberts, 1969; Roberts and Stirling, 1971; Roberts and Smythe, 1974; Roberts and Blight, 1975; Roberts, 1975; Roberts and Hayes, 1977) have studied mechanoreception in the early tadpole stages of Xenopus laevis (stages 21 - 41).

Roberts (1969) found that in early tadpoles, prods to the skin with a blunt pin led to long duration (60 - 300 msecs. in different animals, but constant in any one animal) skin impulses being evoked. These skin impulses have been found to be present up to stage 41, after which they become difficult to record and thus their presence is uncertain (Roberts and Stirling, 1971). Roberts and Stirling (1971) point out that it is most likely that the skin impulses are propagated by "direct current flow from cell to cell."

Roberts and Smythe (1974) suggest that in newly hatched Xenopus laevis tadpoles there are two tactile sensory pathways. One involves the skin impulse and is sensitive to stronger mechanical stimuli. Sensitivity occurs over the entire body surface and begins at stage 24/25. These skin impulses have been shown to evoke muscular responses, probably by the skin impulses spreading from cell to

cell and thereby contacting a Rohon - Beard cell neurite which can then propagate the information back to the central nervous system and cause the response (Roberts and Stirling, 1971).

The other sensory pathway is responsive to light touch stimuli and is not associated with the skin impulse. It begins at stage 26 but then sensitivity is only present at the most cranial region of the myotomes from which it later spreads out over the rest of the body surface with development. Roberts and Smythe (1974) suggest that this light touch sensitivity pathway is associated with Rohon - Beard cells.

Thus Roberts and his coworkers have studied the initial development of mechanoreception associated with Rohon - Beard cells. Since Rohon - Beard cells are only present at trunk levels (Hughes, 1957) it is unlikely that a similar form of mechanoreception will be encountered in the tentacle skin. However, skin impulses may be present. In addition Rohon - Beard cells have small unmyelinated axons with naked nerve endings in the skin, but there may be a difference between the sensory responses monitored from these and those from the 'd' fiber population of Catton (1958) since the Rohon - Beard cells can be possibly regarded as specialised transiently present receptors.

Roberts and Blight (1975) have attempted a correlation between function and structure of non-myelinated free nerve endings in the cement gland of Xenopus laevis tadpoles. They found that these small diameter, slow conducting neurones are involved in the inhibitory control of swimming. These fibers would appear similar to the pain fiber population of Catton (1958) and Sprau (1976) but they respond to non - noxious forms of stimulation. Roberts and Hayes (1977) point out that the hypothesis that unmyelinated nerves which terminate without specialised end structures are concerned with pain has been criticised by various workers, for example Lele and Weddell (1956) and Iggo (1966).

It is possible once again, that these small fibers of Roberts and Blight (1975) are extremely specialised transients to a trans-

ient structure (the cement gland) and are not indicative of the presence of one of the adult fiber populations, viz., the 'd' fiber population.

Whitear (1974) shows that nerves entering frog skin contain both somatic and autonomic components. The investigations of Paterson (1939); Fabian, Hanrahan, Marks and Coombe (personal communication) and Ovalle (1976) make no mention of the presence of glands or muscle associated with the skin of the tentacle. Thus it would seem that only somatic sensory neurones are present and no autonomic component.

From the above it can be appreciated that gaps exist in the understanding of the development of mechanoreception generally, and that mechanoreception has not been studied in the later stages of development in Xenopus nor in the adult. No information is available as to the order, if any, in which the four tactile nerve populations develop, and also no information is available of the effects of each mechanoreceptor population on the behaviour of the animal. Also no work has been done on the neurophysiological responses of Merkel cells in amphibian skin or their contribution to the behaviour of the animal.

Indeed, prior to this study no neurophysiological investigation of the sensory function of the tentacles has been reported.

I. 2 Methods and Materials.

I. 2. I Electron Microscopy.

The purely sensory deep ophthalmic nerve and the sensory part of the mandibular nerve, that is the part distal to the motor branches to the mandibular muscle group, of tadpoles of stages 56 and 57/8 were dissected out under cold saline and then fixed for two hours in formaldehyde/gluteraldehyde fixative (Appendix I). These stages were chosen since at these stages development of the tentacle is complete, and it was these stages that were most

generally used in the physiological investigations.

The nerves were then washed in cacodylate buffer (Appendix 2) and then post-fixed in osmium tetroxide (Appendix 3) for thirty minutes in a fume cupboard. This was then followed by washing in water, dehydration in 100% acetone for thirty minutes, embedding in araldite (Appendix 4) in plastic containers, and finally baking in an oven for forty - eight hours at 60°C. Light gold to silver sections of the nerve were then cut using a glass knife and a Porter - Blum ultramicrotome MT-2. These sections were picked up on a 300 mesh copper grid and observed and photographed on a transmission electron microscope.

I. 2. 2 Physiology.

Tadpoles were killed by destroying the brain with a sharp needle. The tadpole was then staged according to the classification of Nieuwkoop and Faber (1967). The tadpole was pinned onto the bottom of a transparent dissecting dish (Sylgard 184 encapsulating resin; Dow Corning) with a number of minuten insect pins.

Using a Wild M5 dissecting microscope with transmitted lighting, either of the two sensory nerves (Fig. I) could be exposed by removing a square of skin from the relevant dorsal part of the head with a sharp needle and fine forceps.

With the skin removed both sensory nerves could be cut close to their emergence point from the brain and cleared from the underlying cartilage and connective tissue. In the case of the mandibular nerve the various eye muscles, under which this nerve runs, had to be removed before the nerve could be further cleared. More distally the mandibular nerve requires to be dissected out from the mandibular muscle through which it runs.

Sensory information propagated back to the brain from the tentacles via the mandibular or deep ophthalmic nerves was monitored by means of 0,04 mm diameter nichrome wire electrodes. These electrodes were positioned by means of Prior micro - manipulators.

Once the nerve had been wrapped around the electrode, the saline (Appendix 5) was removed with a large syringe until the region of contact between the nerve and the electrode was exposed. The electrode/nerve contact was then covered with a mixture of medicinal paraffin and commercial vaseline (Appendix 6) to prevent dessication. In this way sensory discharges could be monitored for up to 6 hours.

Sensory nerve impulses were first amplified by Grass P5II preamplifiers and then displayed on a Tektronix 5440 oscilloscope fitted with a Nihon Kohden PC - 2A continuous recording camera for filming purposes. Kodak 35 mm Cineflure film (green sensitive) and Kodak photographic paper were used.

In order to stimulate single receptor units a short piece of human hair was used. A strand of hair with a 'split end' was selected and then trimmed so that only the finest part of the hair was left. This was glued into a glass capillary tube attached to the cone of a 8Ω, 1W loudspeaker by means of a section of a plastic syringe. Varying duration and amplitude voltage driver pulses could then be applied to the loudspeaker from a Grass SD9 stimulator. By simultaneously monitoring the applied voltage pulses on the oscilloscope and the sensory discharges it was possible to observe which movements of the hair caused responses from the tentacle.

To immobilise the tentacle during prodding the tentacle was pushed into a fine, deep groove in a piece of plastic with holes through it so that it could be pinned to the bottom of the dissecting dish.

I. 3 Results

I. 3. I Electron microscopy.

Due to the comparatively large diameter of the nerve bundles in relation to the grid square size it was not possible to observe a cross section of the nerve in its entirety. Instead, just portions



Figure 3. Compound action currents monitored from
A. The deep ophthalmic nerve.
B. The mandibular nerve.
following supramaximal electrical stimulation of the tentacle. Calibration; I msec.
The stimulus artefact is visible at the foot of the upward curve indicating the point of stimulation

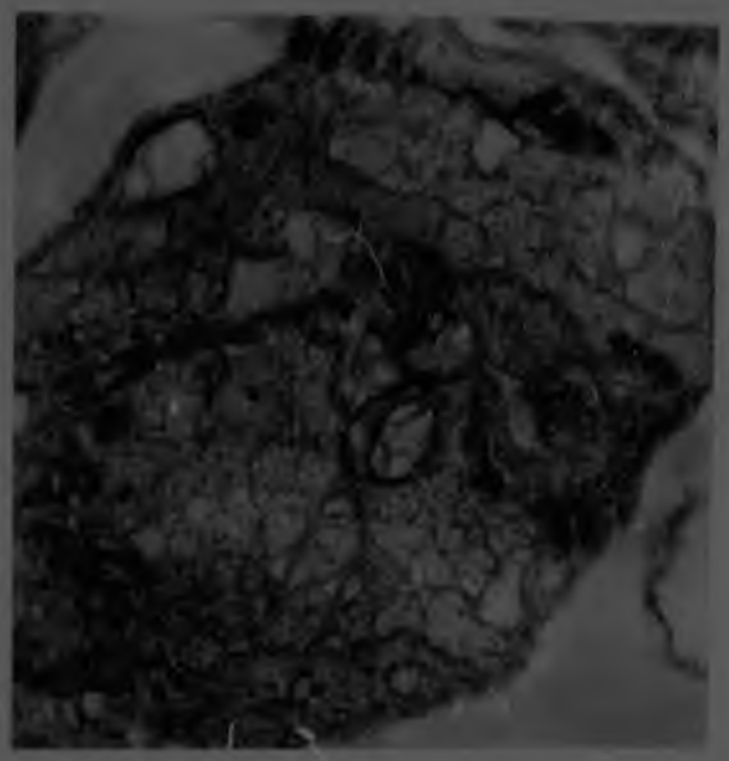

Figure 4A. Transmission electron micrograph of a cross-section of a bundle of unmyelinated neurones in the mandibular nerve of a stage 57/8 tadpole.

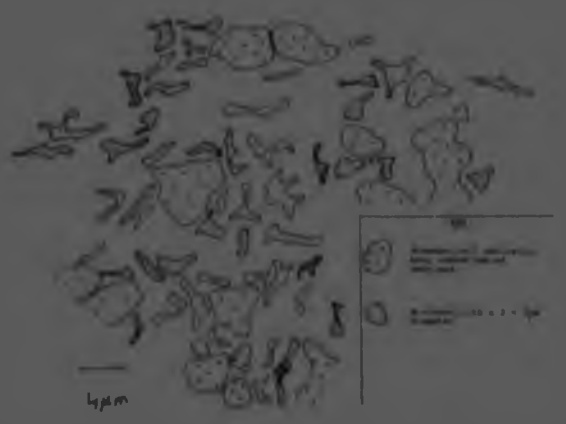

Figure4B. Composite diagram drawn up from electron micrographs of a cross section of the deep ophthalmic nerve of a stage 57/8 tadpole.

of sections at different positions along the nerve were studied.

Since the whole nerve bundle could not be assumed to be circular it was impossible to estimate with any accuracy the number of neurones present in the whole nerve. Serial sections are therefore required so that by observing a number of such sections some overlap between sections can be observed in all cases. Using these a composite picture could be drawn up from all the areas of nerve. This ideal was not realised in the present study.

Both myelinated and unmyelinated neurones were observed in both the mandibular and deep opthalmic nerves proximal to the base of the tentacle.

Conduction velocity experiments (Fig.3), where an electric shock was applied to the tentacle and compound action currents were monitored from both the deep opthalmic and mandibular nerves, confirmed that both myelinated and unmyelinated neurones entered into the tentacle. In each case the first peak had conduction velocities expected for myelinated fibers (2,5 to 9,2 m/sec at 20°C) while the second peak had conduction velocities expected for unmyelinated fibers (0,16 to 0,38 m/sec at 20°C).

In each nerve the number of unmyelinated neurones far exceeded the number of myelinated neurones. No quantitative data is available since the unmyelinated neurones were not always clearly visible and also the whole nerve could not be viewed. However, it is estimated that they outnumbered the myelinated neurones by a factor of at least four.

In all the sections the unmyelinated neurones were all very small, the majority being less than 0,5 μm in diameter. All were found to occur in bundles, each bundle enclosed by a Schwann cell. Comparatively few Schwann cells were found to be present and each enclosed a large number of unmyelinated fibers (Fig. 4A). In all the sections observed there appeared to be no distinct spatial arrangement of unmyelinated fibers in relation to the myelinated fibers. The two appear to occur randomly within the nerve bundle (Fig. 4B).

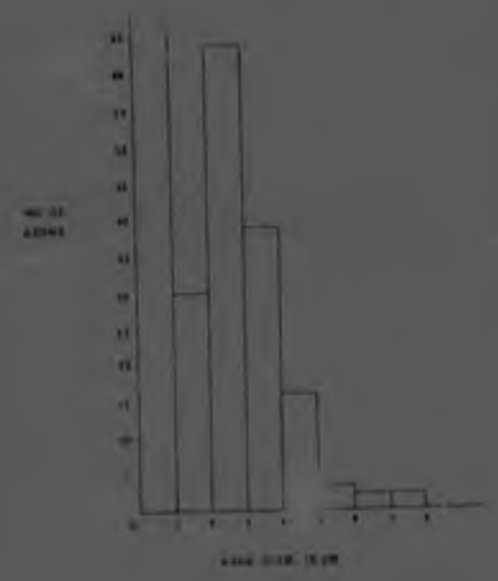

Figure 5A. Histogram showing neurone size composition of the deep opthalmic nerve.

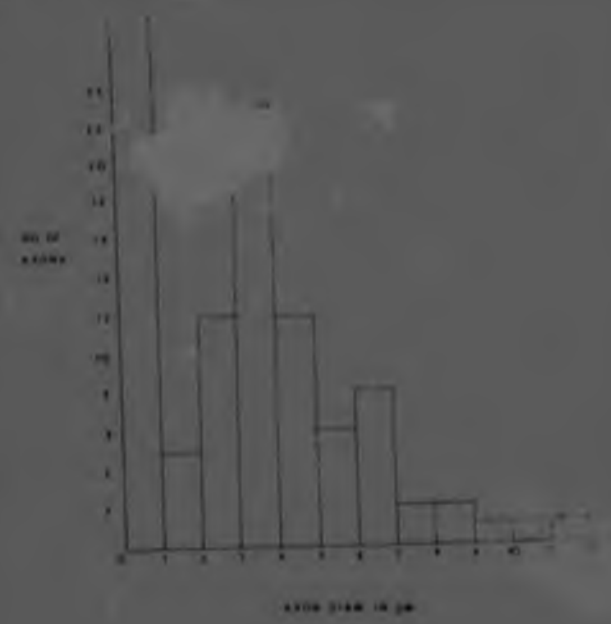

Figure 5B. Histogram showing neurone size composition of the mandibular nerve.

The diameters of all myelinated and unmyelinated neurones which were clearly visible were measured by initially finding the circumference of the neurone by laying a fine piece of thread around them, and then converting this circumference to a diameter. Plotted on a histogram these measurements revealed discrete peaks of axonal size In the deep ophthalmic nerve these peaks occurred between 2 - 3 μm and 0 - I μm (Fig. 5A), while in the mandibular nerve the peaks of axonal diameter occurred between 3 - 4 μm and 0 - I μm (Fig. 5B).

In the deep ophthalmic nerve the two peaks seem to correspond to the peaks of the 'c' and 'd' fibers found by Spray and Chronister (1974) (Fig. 2). However, there is no trace of the other peaks found, that is one at 5 - 6 μm and one at 9 - 10 μm. Due to the apparent randomness of the distribution of the fibers of the two peaks present (Fig. 4B) and the fact that a number of portions of sections were observed, it is unlikely that these fibers are in fact present but bunched together and obscured by the grid in all the sections studied. In addition these fiber populations would have shown up in the conduction velocity experiment if they were present.

The peak between 0 - I μm in the mandibular nerve corresponds to the 'd' fiber peak of Spray and Chronister (1974). However, the peak between 3 and 4 μm is confusing since it has not been previously observed. This peak of axonal diameter is present in all the sections observed if they are considered individually. Thus they are unlikely to be the result of some sampling error. The randomness in the position of the neurones would also tend to support this.

Since this population is definitely present and does not correspond to any of those of Spray and Chronister (1974), who make no mention of Merkel cells, it is possible that it is these fibers that innervate Merkel cells.

It is also possible that these small myelinated neurones are similar to those responding to painful stimulation of toad skin which were described by Maruhashi et al (1952).



Figure 6A. Multi - unit sensory discharges recorded from the mandibular nerve following rapid short strokes of the tentacle causing as little bending as possible. Calibration; I sec.



Figure 6B. Multi - unit sensory discharges recorded from the deep ophthalmic nerve in response to one long tentacle stroke with as little movement as possible. Calibration; I sec.



Figure 7. Sensory responses monitored from the mandibular nerve following manual bending of the tentacle. Note 'passive return phase' response at 'a'. Calibration; 1 sec.



Figure 8. Sensory responses monitored from (A) the mandibular nerve, and (B) the deep ophthalmic nerve following crushing of the tentacle. Arrows indicate estimated start and end of crush. Calibration; I sec.



Figure 9. Sensory responses from the deep optholmic nerve following a short stroke which resulted in tentacle bend.ng. Initially (c) a multi - unit sensory discharge is monitored which includes receptors responding to bending of the tentacle. Later, after the stroke is complete, only the receptors at sites of bending respond irregularly as the tentacle returns to its original position (b).
Calibration; I sec.

Using criteria of fiber size therefore, the deep ophthalmic nerve could contain neurones propagating information on pain, vibration or cold. On the same basis, the unmyelinated neurones of the mandibular nerve could convey information on painful stimuli, and myelinated neurones information from Merkel cells or painful stimuli.

I. 3. 2 Physiology of the tentacle innervation.

I. 3. 2. I Gross responses of the tentacle.

Following strokes of the tentacle with a glass rod which caused as little tentacle movement as possible, a typical multi-unit sensory discharge can be evoked in either of the two nerves innervating the tentacle, that is the mandibular (Fig. 6A) and the deep ophthalmic nerves (Fig. 6B). Similar sensory discharges can be monitored from both nerves following manual bending of the tentacles (Fig. 7); injecting water currents onto the tentacles from an anterior or lateral position which resulted in tentacle bending, and either cutting or crushing of the tentacles (Fig. 8). During such discharges there was never any indication of skin impulses as described by Roberts (1969).

In most cases a stroke along the length of the tentacle resulted in each sensory unit (identified by action potential amplitude) only responding once, or a few times at most, indicating that the units stimulated in this way are extremely rapidly adapting or have minute receptor fields. A technical problem of interpretation is introduced however, by virtue of the fact that action potentials are compounding, rendering amplitude discrimination unreliable. However, observations using fast sweep speeds on the oscilloscope confirmed that the units are in fact rapidly adapting and that the observations were not merely an artefact of compounding.

In cases where stroking of the tentacle resulted in it bending somewhere along its length, it was observed that certain sensory units continued to fire after the completion of the stroke (Fig. 9).

This discharge could be observed to continue until the tentacle returned to its original position and became stationary. The return of the tentacle to its original position is due to the rigidity of the cartilage rod of the tentacle. Units responding during the passive 'return phase' are probably units situated at the sites of bending of the tentacle and are responding as the tentacle slowly bends back to its original position. These units do not respond with a steady impulse frequency as the return of the tentacle to its normal position is not usually a smooth one but can be observed to be jerky.

Similar discharges can be observed during the passive return of the tentacle to its normal position following bending of the tentacle (Fig. 7). Bending was carried out by first crushing the tentacle near its tip with fine forceps and then grasping the region distal to the crush and hence insensitive to mechanical stimulation, and then pulling the tip laterally and caudally. The tentacle tip was then released and the tentacle was allowed to return passively to its normal position. In all cases the tadpole was positioned in such a way that the tentacle did not come into contact with the floor of the dissecting dish during such manoeuvers.

During the active phase of the bend, each sensory unit which did respond seldom discharged more than once. Again, observations using fast sweep speeds on the oscilloscope showed that rapid adaptation was being observed and not an artefact due to compounding. This further illustrates the rapidly adapting nature of the tentacles' mechanoreceptors. If the tentacle was held in a bent position without any movement of the tentacle occurring then a period of sensory quiescence occurred. There was no evidence of more slowly adapting tonic receptors being present. This finding was confirmed in the cases of all the single receptor units analysed later (I.3.2.2). Once again the discharges observed during the passive return after the bend were probably phasic receptor units situated

at the sites of bending and responding to each of the observable jerky movements the tentacle made back to its original position.

The sensory discharges in both nerves following a crush of the tentacle (Fig. 8) appear to be very similar to those following stroking or bending in that a multi-unit sensory discharge occurred. An expected difference which is present is the larger number of receptor units responding to a crush. Naturally a crush of the tentacle would result in both the branches of the mandibular and deep ophthalmic nerves being crushed, leading to firing of all the neurones in each branch at the crush point. Thus more nervous activity should be monitored during a crush than during localised bending or stroking when only part of all the neurones within the tentacle can be expected to discharge impulses. This holds only if the crush is fairly near the base of the tentacle as neuronal branching results in fewer and fewer neurones being present the closer one gets to the tip (Fabian, Hanrahan, Marks and Coombe, personal communication).

Part of this increase in the number of units responding during a crush must be attributed to the fact that no matter how carefully the crush is carried out, both stroking and especially bending stimuli would be present, the latter acting on receptors proximal to the crush point. Therefore sensory discharges in response to these would be expected to be superimposed on those of the crush. This can be seen in figure 8B where receptors still continue to fire after the crush. In this case the crush led to tentacle movement, with the result that after the crush it returned passively to its original position with a resultant irregular sensory discharge, probably from the units at the sites of bending.

There was never any clear indication of any high frequency pain responses during a crush as would be exrected from the findings of Adrian (1926, 1928) and Hogg (1935). The possibility does exist however, that such small amplitude, high frequency responses were present but were not detectable due to the obscuring influence



Figure IO. Sensory responses recorded from the deep ophthalmic nerve in response to manual tapping on the bench. The taps are of increasing amplitude to 'a', then decreasing amplitude. Calibration; I sec.

of the mass of other sensory unit responses. Also, sustained injury discharges, as can be readily observed in invertebrate preparations, were seldom present. Where injury discharges did occur it was usually in a single receptor neurone and never in more than a few neurones. When present the discharge seldom lasted for longer than twenty seconds.

In some preparations, for each nerve, the tentacles appeared to be more sensitive to stroking in certain areas than others. However, there was never any consistency in the position of these areas and since it was generally found that the whole tentacle surface was very sensitive it was concluded that no extra - sensitive areas are present, but rather that where such areas became apparent it was due to the electrode nerve contact in the particular preparation restricting recording from relatively few fibers only.

The tentacles have been found to be extremely sensitive to mechanical stimulation. This is shown in Fig. 10, the sensory discharge monitored from the deep ophthalmic nerve following manual tapping on the work bench at a distance of about one meter from the preparation. The taps are of increasing intensity up to point 'a' followed by decreasing intensity taps. Corresponding to each tap there is an initial burst of sensory activity from many sensory units. The most likely explanation for this is that it is due to receptor units in contact with the dissecting dish, firing in response to the dissecting dish bumping against the tentacle.

Following this initial burst of sensory activity a number of small amplitude action potentials can be noted. They appear in couplet form in some cases. The activity of these receptor units can most satisfactorarily be explained by proposing them to be at sites of bending of the tentacle during its subsequent vibration at a resonant frequency of decreasing amplitude. This record serves to illustrate the extreme sensitivity of the tentacles to what are probably very small bending motions.

It was found that both the mandibular and deep ophthalmic nerves

innervate the tentacles from their earliest stages of growth (stage 44) when they are merely anterior bumps right up until they shrivel up and become reabsorbed at stage 61. This contra - dicts the earlier observations of Paterson (1939) that initially the tentacles are innervated by a branch of the mandibular nerve and that only later, when the tentacles are diminishing, the deep ophthalmic nerve supplies the innervation. However, it was found that at stage 60 the innervation by the mandibular nerve appeared somewhat reduced as compared with that of the anterior ventral skin near the base of the tentacle. Although no quantitative data is available it seems that the sensitivity of the tentacles to mechanical stimulation does not vary during the stages at which they are present.

The deep opthalmic nerve does not only supply mechanoreceptors of the tentacle but also those of the dorsal aspect of the anterior facial region, the dorsal lip of the mouth and the interior lip region of the buccal cavity. A branch of the deep opthalmic nerve runs to the nares and has been found to propagate sensory information back to the brain following light stroking of the interior of the nares with a fine hair.

Similarly, the mandibular nerve does not only convey mechano-receptive sensory information from the tentacles but also from the anterior facial region to the midline of the mouth on either side, and also the ventral surface of the lip and ventral interior surface of the buccal cavity.

Although no detailed comparison has been attempted it appears that the receptors of the tentacles have very similar, if not identical, characteristics to those of the skin of the facial region, such as a very rapidly adapting response and low threshold.

I. 3. 2. 2 Single unit responses.

Using a fine hair attached to the cone of a loudspeaker to which driver voltage pulses were applied proved to be a useful technique for stimulating single receptor units. The inherent



Figure II. Records showing receptor units being recruited with increasing prods of short duration. The prod amplitude causing the record on the right was a few μm, while those causing the record on the left were approx. 40 μm. The middle record was from prods of intermediate amplitude. For 'a', 'b' and 'c' see text. Calibration; I sec.

problem with using such a technique on a cylindrical area of skin is that a prod to one side results in pressure being exerted on the diametrically opposite part by the supporting medium. In this case it was reasoned that since the area of the hair (approx. $0,01^2$mm. diam.) was so small in relation to the area of the tentacle in contact with the supporting plastic, the force exerted by the hair was considerable compared with that exerted by the supporting plastic during the prod. In addition, the tissues of the tentacle would provide a damping medium for the forces transmitted to the skin on the opposite side during a prod. Thus since the localised force exerted by the hair was greater than the more diffuse force of the supporting medium resulting from a prod, receptors below the hair could be stimulated with supra - threshold intensity without evoking responses from receptors on the opposite side.

With square wave driver voltage pulses it was found that forward movements of only a few micrometers by the hair tip were sufficient to evoke a response if the tip of the hair just dimpled the skin surface. This further illustrates the extreme sensitivity of the tentacle skin to light mechanical stimulation.

Catton (1970) points out that the lowest thresholds are usually found in skin overlying less resilient tissue, such as bone. In toad skin, mechanical displacements necessary to evoke a response were found to be 10 μm in skin overlying bone and 150 μm in skin overlying less resilient tissue. Thus the mechanical displacement found to be sufficient for the tentacle skin is extremely small bearing in mind that the underlying tissue is very soft indeed. The skin never overlies the cartilage since there is always other tissue interposed, especially connective tissue.

As the forward prod of the hair was increased up to about 40 μm it was found that more and more receptor units responded (Fig. II). This may have been due to the presence of a number of overlapping receptor unit fields, and that the thresholds for the evocation of a response in the receptor units varied.

In Fig. II the units responding at 'a' and 'b' are most likely to be those directly beneath the hair tip. The time discrepency between 'a' and 'b' can be accounted for by proposing that the units responding at 'a' and 'b' have different diameter axons and thus different conduction velocities. Hence, if the 'b' unit had a smaller diameter axon than that at 'a' then its action potential, following stimulation, would only arrive later at the recording electrode site.

The responses of the receptor units at 'a' and 'b' appear to be constant in all three different amplitude prods. However, in the high amplitude prod, the one on the left in Fig. II, an additional two receptor units were present at 'a'. These two receptor units fired constantly at this position for all prods of this amplitude. This strongly suggests that these receptor units, which only came in at higher amplitude prods, are in fact receptor units present immediately below the hair tip but have higher thresholds for the evocation of a response. Although this is the most likely explanation, it is also possible that these two extra units responding at 'a' could be elements of the 'c' group of responding receptor units.

The receptor units responding at 'c' in Fig. II are probably those receptor units situated at sites of bending caused by the previous prod. One would expect such responses after a delay because it would take time for the tentacle to bend following a prod. They could also be thought to be high threshold receptor units situated below the prodding hair tip, but with larger diameter axons than 'a' and 'b' receptor units. This would appear unlikely though, since their response is apparently incon - sistent and one would expect a consistent response if these units were situated directly beneath the prodding hair. A clear indication of the lack of consistency is the appearance of a large spike ampli - tude unit at the medium amplitude prod at 'c' which then disappears at the high amplitude prod and at the low amplitude prod.

With increasing amplitude prods it was observed that tentacle move - ment resulted at locations on the tentacle further removed from the dimpling at the point of stimulation. Thus it is possible that the responses ('c' responses in Fig. II) were being evoked from receptor units not being stimulated directly by the hair, but rather indirectly at the sites of bending further removed from the point of stimulation. This idea is supported by the finding that a number of identical large amplitude prods seldom resulted in the same units responding all the time at 'c' although the response of certain units (at 'a' and 'b') was constant to each prod. The units responding inconsistently at 'c' were probably those at sites of bending of the tentacle. It is unlikely that the tentacle returned to its exact original position after a large amplitude prod and thus sites of bending along the tentacle would be expected to vary from prod to prod. The consistently responding units at 'a' and 'b' were most likely those directly beneath the hair tip.

The delay of receptor unit response as can be observed in Fig. II (from previous prod to 'c') could be evidence of lateral spread of the stimulus. That is, with larger amplitude prods more and more receptor units were stimulated since the area of the skin dimpled by such prods grew larger as the amplitude of the prods increased. Thus receptor units may have been present close to the point of stimulation and were only recruited when the hair tip caused dimpling of the skin where the receptor unit's field terminated. Since the dimplings of the skin appear to be consistent with each prod this explanation does not account for the inconsistency of response of various units. Rather the delay of receptor unit responses (that is from previous prod to 'c') should be attributed to the delay between stimulation and threshold bending occurring

A further possibility is that with large amplitude prods the neurones within the tentacle were stimulated directly. Julian and Goldman (1962) found that frog myelinated axons respond to mechanical displacements of 2 - 5 μm. Thus each large amplitude prod may have been directly evoking a response in differing neur - ones within the nerve bundle. In the majority of experiments the prod amplitudes were kept as small as possible in an attempt to avoid this possible fac to-



Figure I2. Responses monitored from the deep ophthalmic (A) and mandibular nerves (B) in response to prods to the tentacle caused by square wave driver voltage pulses. Note that the advance phase of the stimulus in A causes both 'a' and 'b' neurones to fire, whereas the return phase elicits only the small amplitude spike 'b'. Calibration; A - I sec. B - 0,I sec.

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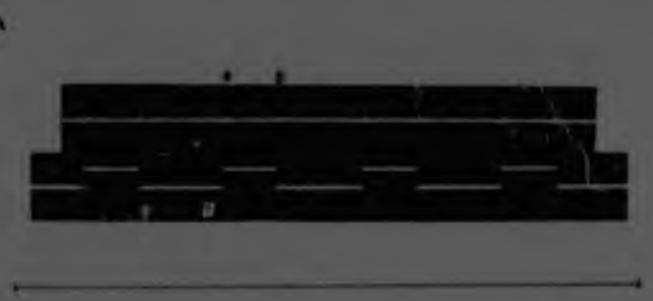

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If the tentacle skin could be removed and then prodded on a smooth flat surface, the reason for the observed stimulus response characteristics could doubtless be revealed. However, this was not possible due to the technical difficulties associated with such fine dissection.

Although in the above example it is difficult to conclusively show overlapping receptive fields at one spot, it is possible to do this if very small amplitude prods are used which do not cause any tentacle movement. With such low amplitude prods it was often found that a prod could evoke a response from two receptor units in the same nerve (Fig. I2). Similarly it was often found that a prod could evoke a response in both nerves, suggesting that the field of innervation of both nerves overlap considerably.

In most cases it was found that if one slowly increased the amplitude of the prod, receptor units of the deep ophthalmic nerve responded first and the mandibular nerve receptor units only responded after a further increase in prod amplitude. This suggests that there is some difference in thresholds between overlapping receptor units of the two innervating nerves, albeit very small.

The slight increase in prod amplitude required to evoke a response in the mandibular nerve often also resulted in further units responding in the deep ophthalmic nerve. This suggests a difference in thresholds of receptor units in one nerve since such slight increases in prod amplitude did not result in any tentacle bending or an observable increase in skin dimpling around the hair tip, making it unlikely that adjacent receptive fields were being activated.

Differences in the adequate stimulus for elicitation of a response in two receptor units is shown in Fig. I2. Both receptor

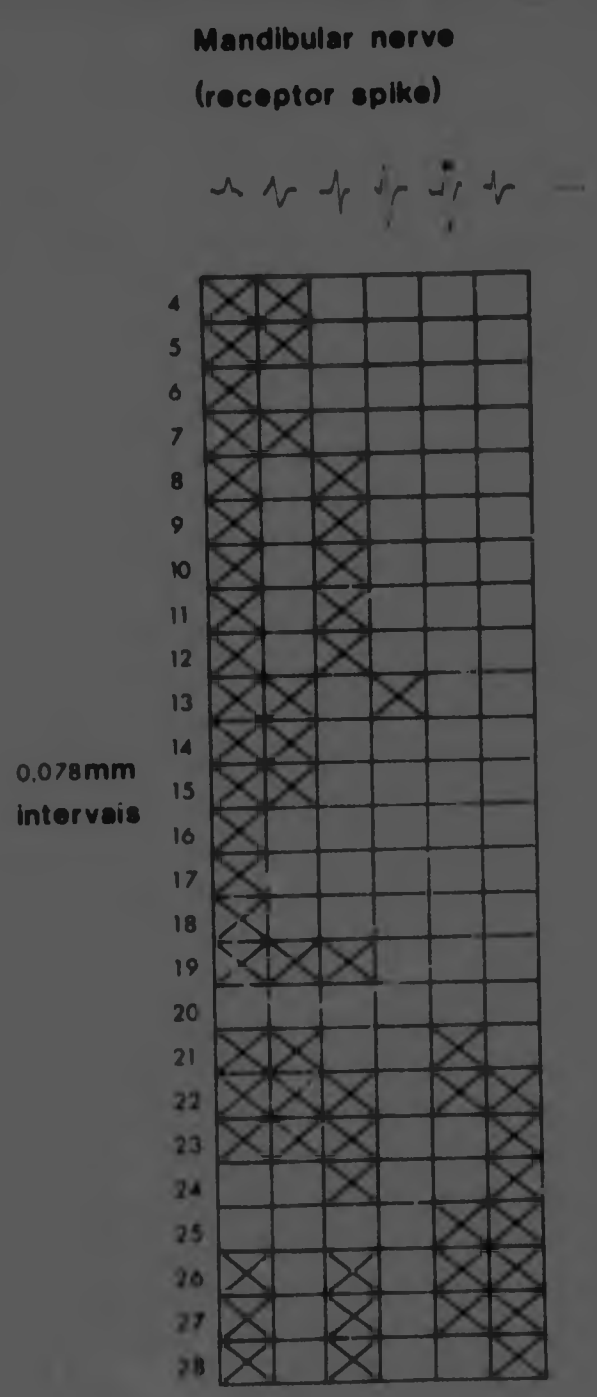

Figure 13A. Receptor spikes monitored from the mandibular nerve in response to prods of the tentacle at 0,078 mm intervals along its length. Six receptor units could be distinguished on the basis of spike amplitude and waveform. Sample spikes are shown. Calibration; I msec.

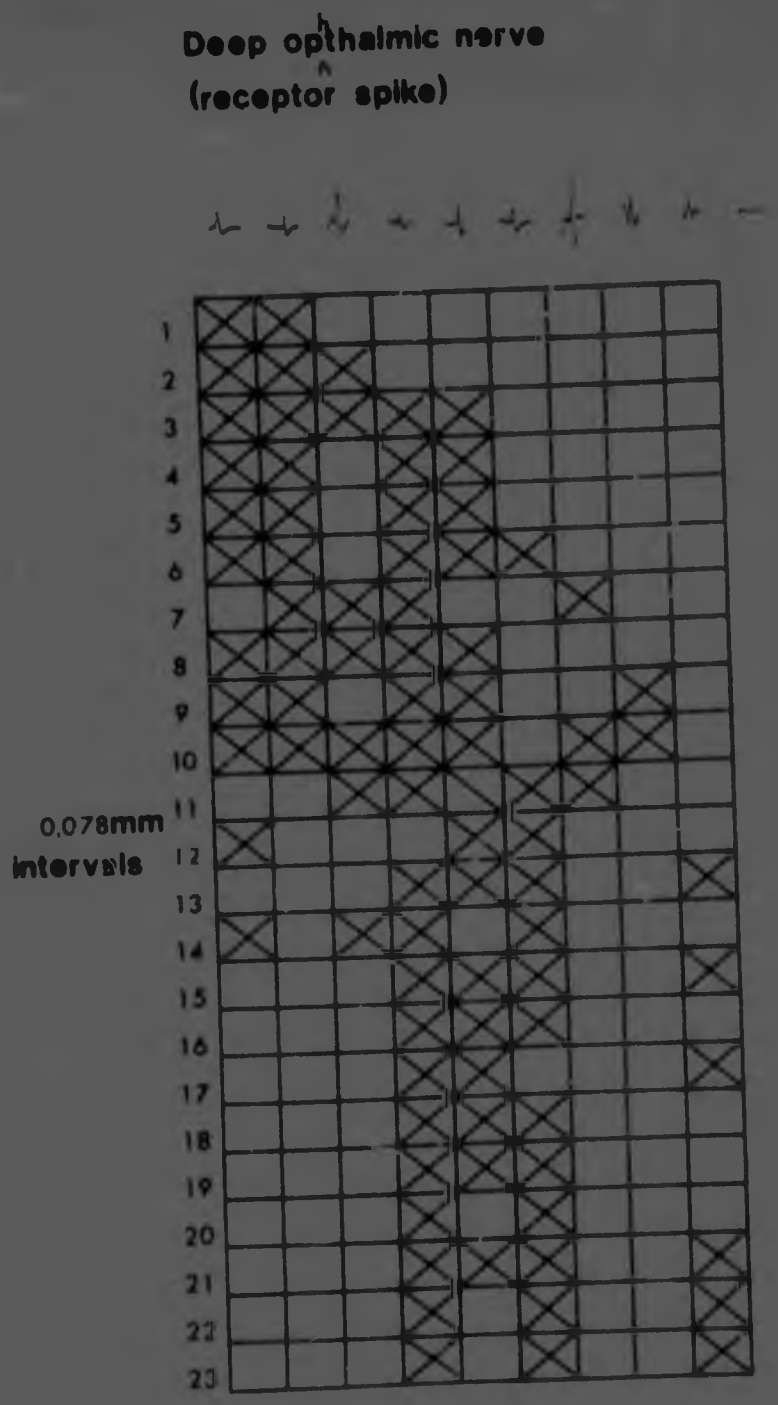

Figure 130. Receptor spikes monitored from the deep ophthalmic nerve in response to prods of the tentacle at 0,078 mm intervals along its length. Nine receptor units could be distinguished on the basis of spike amplitude and waveform. Sample spikes are shown. Calibration; I msec.

units in the deep ophthalmic nerve respond to the advance phase of the prod at 'a', but when the hair is withdrawn only the smaller amplitude unit responds. In all cases where such responses were monitored an increase in stimulus intensity could not induce a receptor unit to respond to a particular phase of the prod if it did not do so at low intensities. This confirmed that the receptor units present respond to different phases of the mechanical stimulus rather than it being an effect of stimulus intensity.

Fig. I2 also shows the extremely phasic nature of the receptor's response most spectacularly. There is no evidence of any tonic response during the maintained phase of the prod. This was found to be typical of all prods to all areas of the tentacle.

As far as the dimensions of the various receptive fields is concerned, valuable information was gained by prodding the tentacle at various points along its length. At each position the prodding hair was placed in contact with the tentacle and then the amplitude of the prod was adjusted until it was just below the level that caused movement in surrounding regions of the tentacle. This hopefully avoided receptor units adjacent to the point of stimulation being stimulated. Within this constraint, increasing the intensity of the stimulus as much as possible should have ensured that stimulation was supra - threshold for all units immediately beneath the hair tip.

Fig. I3 shows typical results obtained from the mandibular and deep ophthalmic nerves (I3A and I3B respectively). Due to the cylindrical shape of the tentacle and the necessity of pushing the tentacle into a groove to prevent movement, it was not possible to measure widths of receptive fields, but rather only lengths along the tentacle where it protruded from the groove.

What is initially striking from these results is that fewer receptor units are present in the case of the mandibular nerve than for the deep ophthalmic nerve. The most likely reason is that responses in both nerves were photographed simultaneously for



Figure 14. Diagramatic representation of two possible receptive fields. A - irregular but continuous. B - scattered, non - continuous.

each prod at each position, and since it has already been observed that the mandibular receptor units have higher thresholds than at least some of the deep ophthalmic nerve receptor units, there should be fewer mandibular nerve receptor units responding to the same prod than for the deep ophthalmic nerve. However, the possibility still exists that similar large numbers of receptor units overlap at a particular spot for the mandibular nerve, but that the stimulation supplied was below threshold for them. Perhaps threshold stimulation for these proposed units would be of such an amplitude as to cause bending of the tentacle, thus their presence was not detected. The only way to test this possibility would be to perform a histological analysis. The ratio of the number of fibers innervating the tentacle to tentacle skin area for each nerve should be computed. If the ratios were similar for each nerve then the above hypothesis for each nerve would appear likely.

The lengths of the receptive fields measured varied considerably from 0,1 mm right up to 2,0 mm. Since there was no grouping of receptive field lengths, it was concluded that receptor units cannot be further characterised by the length of their receptive fields. It may be possible to group receptor units according to receptive field areas, but this was not possible to ascertain since measurement was only possible in one plane.

For both nerves it was found that for most of the receptor units response could only be evoked intermittently along the tentacle. This finding can be explained in either of three ways: firstly it is possible that the shapes of the receptive fields are irregular, although continuous, as shown in Fig. I4A. The second possibility is that a receptor unit field comprises scattered, non - continuous areas of skin (Fig. I4B). If it were possible to prod over a comparatively large area of skin then the true situation, with respect to these two alternatives, could be ascertained. The third possibility is that within a receptive field there is a variation of threshold from part to part. If this were

the case it is possible that as the stimulating hair was moved down the tentacle it alternately moved into regions of high and low thresholds leading to responses only being intermittently elicited in the low threshold regions of the receptor field. Although this variation in threshold within a receptive field has been previously observed (Roberts and Blight, 1975) it has never been reported that threshold can be high, low, high, low, etc. across a receptor unit field as would appear here.

It was not possible to test whether the interrupted response pattern was due to variation of threshold within the receptive field. Due to the small amplitude of the prods delivered, coupled with the fact that it is not really possible to apply the hair tip with identical pressure to different parts of the tentacle because of the tentacle's shape and its low resilience, it means that it is not possible to stimulate a number of spots on the tentacle identically. Slight variations must always occur which would not allow reliable results to be obtained concerning thresholds within a receptive field.

I. 3. 2. 3 The Merkel cells of the tentacle.

In all the results so far presented there has been no indication of the presence of any Merkel cell responses such as those described by Iggo and Muir (1969). In all cases no high frequency response was ever observed in response to traversing a smooth probe across the tentacle surface. Similarly no static response was ever monitored, no matter the amplitude of the displacement of the stimulus. This strongly suggests that in the skin of the tentacles, although there are Merkel cells present (Ovalle, 1976) they do not appear to function as mechanoreceptors; at least not in the previously described manner of Iggo and Muir (1969). However, it may be that since the morphology of the touch domes differs from merely having Merkel cells scattered throughout the tentacle skin, the physiological properties may differ. Thus further investigations were carried out.

Figure 154. A record of the responses of the mandibular (top trace) and deep ophthalmic (bottom trace) nerves in response to a stroke of the tentacle which caused tentacle bending. This film was taken just prior to Mg^{++} application. The amplifier gain is half that of 153B and C
Calibration; 1 sec.



Figure 15 B. A record of the responses of the mandibular (top trace) and deep ophthalmic (bottom trace) nerves to a stroke of the tentacles which caused bending. This film was taken 9 mins. after $Mg.^{++}$ application. Note the relative decrease in the number of receptor units responding in the mandibular nerve as opposed to the deep ophthalmic nerve. The receptor units responding in the mandibular nerve only respond during the middle of the stroke, and in an irregular feshion suggesting that they are unmyelinated neurones responding to tentacle bending.
Calibration; I sec.

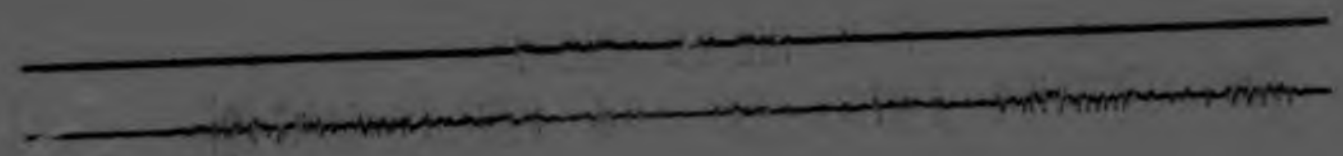

Figure 15C. A record of the responses of the mandibular (top trace) and deep ophthalmic (bottom trace) nerves to a stroke of the tentacle which caused bending. This film was taken 19 mins. after $Mg.^{++}$ application. Once again, the relative decrease in the number of receptor units responding in the mandibular nerve as opposed to the deep ophthalmic nerve. The receptor units responding in the mandibular nerve only respond during the middle of the stroke, and in an irregular fashion, suggesting that they are unmyelinated neurones responding to tentacle bending. Calibration; I sec.

On the assumption that the association between a Merkel cell and a neurite involves synoptic transmission (Munger, 1965), it was considered worthwhile to investigate the effects of altering the Ca^{++} and Mg^{++} ratio of the saline used. Assuming that Merkel cell feedback is susceptible to this form of experimental manip - ulation, it could provide a means for distinguishing that part of the overall sensory response, if any, that is due to Merkel cells.

In such experiments the dissection and the placement of the electrodes was carried out under ordinary saline (Appendix 5). The saline was then emptied from the dissecting dish and fresh saline with a 0,05M Mg^{++} and 0,009 Ca^{++} content was poured in. The mandib - ular nerve electrode was then connected up to a stimulator. Stimulation of this nerve led to contractions of the mandibular muscle group. Once these contractions could no longer be evoked it was evident that the Mg^{++} had taken effect and caused synaptic blocking. The sensory responses of the tentacle could then be monitored. This would be a response neither contributed to or influenced by Merkel cells.

Fig. I5A shows the response monitored from the mandibular and deep ophthalmic nerves following stroking of the tentacle, including an element of bending. The response is typical, that is a multi - unit response for each nerve but with more units responding in the deep ophthalmic nerve.

Fig. I5B and C shows the responses monitored from the same preparation 9 min. and I9 min. after Mg^{++} application. A multi - unit sensory response is still evident for the deep ophthalmic nerve which appears similar to the response monitored in normal saline. But in the case of the mandibular nerve the response is from fewer receptor units than are active in normal saline. This drop in the number of receptor units responding to a stroke was apparent in all preparations tested and seems to suggest that Merkel cells, involving as they probably do, chemical synapses, contribute to the sensory response of the mandibular nerve.

These results however, could equally well be interpreted as a result of the aforementioned higher thresholds for the mandibular nerve receptors, coupled with the fact that no two manually applied strokes of the tentacle can be identical in their intensity.

If this finding that the mandibular nerve possibly contains neurones terminating on Merkel cells is correct, then a great difference is present between the responses of Merkel cells here and in cat and certain primate skin's where they have been described by Iggo and Muir (1969). Firstly the adaptation rate of the phasic part of the responses appears to be far greater - seldom does a response involve more than one action potential as opposed to high frequency bursts. Secondly no static response is apparent. This all tends to confirm Iggo and Muir's (1969) earlier mentioned hypothesis that the physiological characteristics of the touch domes may be dependent on the structure of the corpuscle.

According to Munger (1965) neurites only rarely end on Merkel cells, but rather usually ascend into the epidermis after coming into intimate association with a Merkel cell. Ovalle (1976) has confirmed the synaptic nature of the association in the case of the Merkel cells of the tentacle. Munger (1965) proposed that Merkel cells could conceivably modulate the growth or function of neurites. In the light of the apparent effect of Mg^{++} on the discharge of mandibular nerve units it would appear unlikely that Merkel cells merely subserve a modulating effect on function of the neurite. His other suggestion that they may modulate growth of neurites may still be possible.

I. 3. 2. 4 A comparison of the receptor units of the Xenopus tadpole tentacle and those of Catton (1958) and Spray and Chronister (1974).

The finding that Merkel cells appear to be associated with mandibular nerve neurones probably explains the unexpected population of nerve fibers found in this nerve, with diameters ranging

from 3 - 4μm (Fig. 5B). This population does not fit in with the findings of Spray and Chronister (1974) who found populations with diameters between 0 - 1μm.; 2 - 3μm.; 5 - 6μm. and 9 - 10μm. Spray and Chroni ter (1974) make no mention of Merkel cells being present. It is likely therefore, that the mandibular nerve contains elements of the system described by Spray and Chronister (1974), but in addition contains a comparatively large number of neurones which are not present in their system but are associated with Merkel cells in this system. This finding that some elements of Spray and Chronis er's system, for example a population between 0 and 1μm. and the subpopulation between 2 - 3μm. explains why responses are still present after Merkel cell inactivation.

The two nerve fiber populations observed in the deep ophthalmic nerve appear to be similar to those in the dorsal cutaneous nerve as described by Spray and Chronister (1974) without the large diameter populations of nerve fibers being present. This may be as a result of the tadpole skin still being in a developmental stage. Neurones with diameters of 3 - 4μm. are also present in the deep opthalmic nerve, thus this nerve may also propagate information from Merkel cells, but to a lesser degree than the mandibular nerve.

The fiber populations present in the deep ophthalmic nerve appear to be the 'c' and 'd' populations initially described by Catton (1958). The 'c' afferent fiber population responds to thermal stimulation (Spray, 1976), although it could respond to vibration as originally stated by Cotton (1958). Spray (1976) points out that since heating causes a decrease in the discharge of the receptors while cooling causes an increase in the discharge, they should be called cold receptors. The 0 - 1μm. diameter group which is present in both the mandibular and deep ophthalmic nerves should correspond to the 'd' fiber population and should re nond to nociceptive stimulation of the skin.

With respect to the 'c' fiber population Hensel et al (1960) include the following two criteria for temperature receptors.

Firstly, they must have a static and dynamic thermal sensitivity in the same range as those stimulating temperature receptors in humans. Secondly, thermo - receptors should be relatively insens - itive to mechanical stimulation.

When a small block of ice was placed about I mm. from the tentacle this resulted in an average drop of the temperature of the saline in the tentacle's position of 2,5°C in about five seconds. No neural response was ever monitored from either innervating nerve in response to this. In fact even when the block of ice was placed in contact with the tentacle only a brief burst of sensory activity was observed which was typical of that recorded following a light stroke. This is in marked contrast to the results of Spray (1976). Since it is with cooling that the so - called cold receptors respond with accelerated discharge, if thermoreceptors are present in the tentacle skin some indication of this response should have been easily discernible by cooling the tentacle. A 0,5°C/sec. temp - erature decrease resulted in a linear increase of firing rate from 0 impulses/sec. to IO impulses/sec. in approximately four seconds (Spray, I976). No sign of this form of response was ever observed in the tentacle. Thus since no impulses were ever observed in response to a change in temperature, it was concluded that most probably no dynamic temperature sensitivity was present in any receptors of the tentacle. Similarly, since no spontaneous activity was ever monitored from either sensory nerve, even following changing of the saline in the dissecting dish with cold saline, it can be concluded that most probably no static temperature sensitivity occurs in any receptor unit of the tentacle.

As has already been shown the tentacle skin contains extremely sensitive mechanoreceptors. These mechanoreceptors could be inner - vated by either the small 'd' fiber population or the larger 'c' fiber population. When the conduction velocity of responses to prods along the tentacle were investigated it was found that the conduction velocity was never slow enough to place the receptor

in the 'a' fiber catagory. In all the cases observed prods resulted in responses with conduction velocities of between 2 and 10 m/sec.

This shows that the supposed 'c' fiber population here contains receptors which are extremely sensitive mechanorecep - tors.

Thus it has been shown that no static or dynamic thermal sensi - tivity is present, even though the stimuli used mediated thermal sensations in humans. In addition the supposed 'c' fiber population are extremely sensitive mechanoreceptors. Thus this fiber populat - ion differs markedly from that observed by Spray (1976) and cannot be regarded as a cold receptor fiber population.

Due to the inadequate stimulators available it was not possible to test whether the receptors responded to high frequency vibration as was the case with Catton's (1958) 'c' fiber population. The extracellular spike amplitude for Catton's 'c' fiber population was between 100 and 150 μV. This extracellular spike amplitude was only seldom observed in the tentacle skin preparation. The majority of spike amplitudes were between a few microvolts and 100 μV. The variability in size was continuous. This continuity in amplitudes can be attributed to some large amplitude signals from neurones not in contact with the recording electrode, atten - uating before reaching the electrode and hence giving smaller signals than are in fact present. In this way a smooth spectrum of spike amplitudes would be monitored, as opposed to distinct groups of amplitudes.

In addition to the difference in spike amplitudes observed between the small myelinated nerve population of the tentacle and the 'c' fiber population of Catton (1958), a further difference is that thermal stimulation was not an effective way of evoking a response as was found to be the case for Catton (1958) in his population. Similarities between the two populations are conduc - tion velocities and the fact that they both have a widespread distribution in the skin.

From this it is clear that the small myelinated axon population observed in the tadpole tentacle is not at all similar to the population described by Spray (1976). In addition, certain differences are present between this system and the 'c' fiber population of Cotton (1958), which make it unlikely that they are the same. Thus it would appear that the 2 - 3μm. fiber population described here in the deep ophthalmic nerve is somewhat novel. However, this novelty may be more apparent than real due to the skin of the tadpole still being in a developmental stage. A comparative study on the skin innervated by the deep ophthalmic and mandibular nerves of adult Xenopus would clarify whether the fiber population described here continued after metamorphosis with the same characteristics and hence is a new fiber population, or whether the characteristics of this fiber population change with metamorphosis to coincide with one of the already described populations.

In relation to the proposed pain fibers of the electron microscopy investigation, differences have been found between the physiological responses of these in this preparation and those of Adrian (1926); Hogg (1935) and Catton (1958).

Some similarities are present though. Firstly, the conduction velocities estimated from the various experiments of this investigation have usually fallen between 0,1 and 0,9 m/sec. Although inaccuracies are inherent in measuring conduction velocities, those found here correlate fairly well with the conduction velocities of Catton's (1958) 'd' fiber population; mainly 0,1 to 0,3 m/sec. Secondly, in the nerves innervating the tentacle, impulses are usually completely absent unless the tentacle is stimulated. This is similar to the finding of Adrian (1926) but differs from that of Catton (1958), who found that irregular discharges, which could persist for up to two minutes, were frequently found in his 'd' fiber population.

Although long duration 3 - 5 msec. action potentials were observed occasionally, it was generally found that the actio-

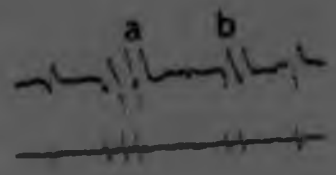

Figure 17. An extract from a record from the deep ophthalmic nerve of a stroke causing tentacle bending. The top trace was from an electrode 0,9 mm proximal to the electrode giving rise to the bottom trace. 'Cross-talk' is apparent in the upper trace, facilitating conduction velocity calculations. At 'a' is probably the response of an unmyelinated neurone (conduction velocity approx. 0,7 m/sec), while at 'b' is probably the response of a small myelinated neurone (conduction velocity approx. 2,3 m/sec).
Calibration; 20 msecs.

potential duration of the small myelinated fibers was very similar to that of the impulses of the tactile, faster conducting neurones (Fig. I7 and I8). This is in contrast to the much longer duration action potentials for this neuronal type found by Hogg (I935) (I5 - 70 msecs.) ; Fessard and Segers (I943)(7 - I8 msecs.) and Catton (I958) (5 - I5 msecs.).

Two major differences were present between the responses of these unmyelinated neurones innervating the tentacle and those unmyelinated neurones associated with pain sensation in amphibians as described by Adrian (I926); Hogg (I935); Fessard and Segers (I943); Catton (I958) and Spray (I976). Firstly these unmyelinated fibers could be found to respond to obviously non - noxious forms of stimulation, and secondly a tonic response was never observable.

Although prodding of the tentacle surface was never found to result in a sensory discharge in unmyelinated neurones, these could be found to respond to stroking of the tentacle (Fig. I7). Stroking in these cases always caused tentacle bending, which was often only very slight. Both stimuli are however, completely non - noxious.

Since the stroking caused bending it is not really possible to define the effective stimulus for these unmyelinated neurones as being stroking or bending. However, stroking and prodding are similar because both involve depression of the epithelial cells and thus shearing forces between cells where the neurones terminate, while bending causes compression on the inside of the bend and stretching between cells on the outside of the bend. These would be the forces present which may or may not trigger the transducer mechanism. Since these forces are effectively at right angles to one another this means that there are fundamental differences between stroking and prodding, and bending. Because of these differences it is most probable that it is bending which is the effective stimulus for the unmyelinated neurones.

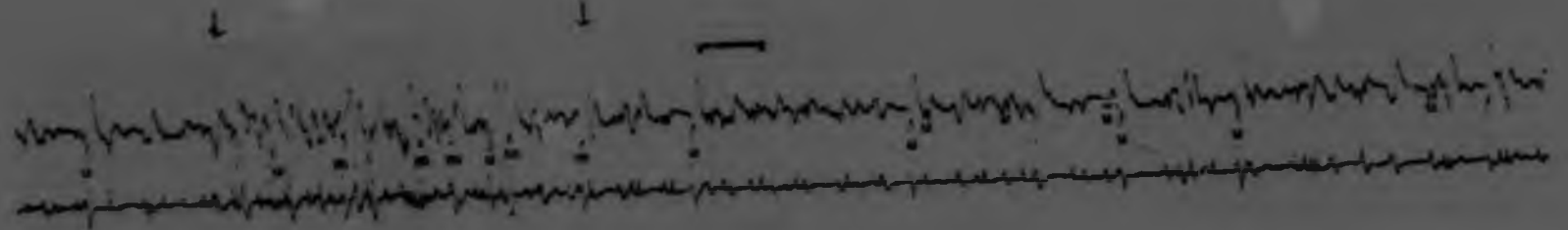

Figure 18. Multi - unit sensory response monitored from the deep ophthalmic nerve with two nichrome wire electrodes, following a crush of the tentacle just proximal to its tip. The electrodes were placed 0,95 mm. apart, with the electrode of the bottom trace distal to that of the top trace. 'Cross talk' was present between the two recording channels. 'u' - probably unmyelinated neurones with conduction velocities less than I m/sec. 'm' - probably myelinated axons with conduction velocities of greater than I m/sec. Calibration; IO msecs. ARROWS INDICATE ESTIMATED START AND END OF CRUSH

Adrian (1926) found that in response to noxious stimulation of the skin, frequency discharges of up to 150 Hz. could be monitored from small unmyelinated neurones. From his records the frequencies observed appear regular. Similarly Hogg (1935) found frequencies of between 30 to 40 Hz., while anything above 15 Hz. was regular. Catton (1958) also mentions a tonic discharge from small unmy - elinated neurones in response to noxious stimulation.

In addition Hogg (1935) found a non - reducible delay of between 500 to 700 msecs. before maximum response occurred in these neurones following noxious stimulation. Also Spray (1976) points out that since Adrian (1926) stimulated touch receptors in addition to pain receptors, if all the impulses occurring before the first 700 msecs. of his responses are ignored, then the activity pattern recorded appears very similar to those of Hogg (1935).

In all the crushes of the tadpole tentacle such maximum tonic responses could never be observed 500 to 700 msecs. after the beginning of crushing (Fig. 8A and B). Rather irregular discharges occurred after the initial high neural activity corresponding to the crush. Such irregular responses do not appear very markedly different from those recorded just prior to the crush when the forceps made contact with the tentacle resulting in bending and stroking stimuli being applied. This is evident in Fig. 18. It would appear likely that the responses monitored after the crush and before the crush are due to the same stimuli, that is bending and stroking or prodding. There is also probably some injury discharge after the crush, but as can be seen in Fig. 18 this is not very prevalent.

In Fig. 18 it can be seen that both myelinated and unmyelinated neurones are responding before, during and after the tentacle crush. The responses during the actual crush are mostly single. This is very unlike the position in invertebrate preparations where injury discharges can be observed to persist for a long time and at high frequencies. Since these injury discharges

appear to be very rapidly adapting in this preparation, with only a few neurones ever having been observed to persist in firing after the crush, it is probable that the responses monitored after the crush are from those units situated at sites of bending of the tentacle following crushing - especially in the case of the unmyelinated neurones which have been shown to be most likely responsive to bending of the tentacle. From the findings of Adrian (1926) and Hogg (1935) one would not expect to find un - myelinated neural responses so soon after the crush, or before the crush if these were typical amphibian pain receptors.

Thus these unmyelinated fibers in the tentacle do not appear to function similarly to those of Adrian (1926), Hogg (1935) and Catton (1958). Rather they appear similar to the responses that Roberts and his various coworkers (Roberts and Smythe, 1974; Roberts and Blight, 1975, and Roberts and Hayes, 1977) observed in Rohon - Beard neurites of Xenopus tadpoles and those unmyelinated neurones terminating in the cement gland of early Xenopus tadpoles.

Once again it may be that the responses are not characteristic because the tadpole skin, where the neurones terminate, is still in a developmental stage. Only a comparative investigation of adult Xenopus skin would resolve this question.

I. 4 Conclusions.

The tentacle is innervated by two distinct neuronal populations in both the mandibular and deep ophthalmic nerves. A O - 1 μm diameter population is present in both nerves, while in the deep ophthalmic nerve an additional one of 2 - 3 μm diameter is also present. The mandibular nerve has an additional population to the small unmyelinated one, with diameters from 3 - 4 μm.

The unmyelinated neurone population common to both nerves and the small myelinated fiber population of the deep ophthalmic nerve were atypical, physiologically, compared to similar diameter fiber populations

found in other amphibian skin preparations (Adrian, 1926; Hogg, 1935; Catton, 1958; Spray and Chronister, 1974, and Spray, 1976). An investigation of the sensory aspects of the mandibular and deep ophthalmic nerves of adult Xenopus is required before any conclusion can be drawn as to whether the populations in the tadpole are atypical because they are new populations which have not been described before, or are the same populations as described before but have different physiological characteristics because the skin of the tadpole is not yet fully developed.

The 3 - 4μm diameter fiber population in the mandibular nerve has been hypothesised to innervate the Merkel cells which Ovalle (1976) showed to be present. This being the case, it has been shown that the physiological characteristics of the Merkel cells in the tentacle vary considerably from those described in mammals by Iggo and Muir (1969). This would be consistent with the hypothesis of Iggo and Muir (1969) that the morphology of the touch domes influences their physiological characteristics.

The tentacles have been shown to be extremely sensitive mechano - receptive appendages, being sensitive to bending (responses in myelinated and unmyelinated neurones), prodding (myelinated neurones only) and stroking (probably only myelinated neurones).

Innervation of the tentacle was found to be present throughout their existence. The receptive fields of the innervating neurones were found to be either continuous but irregular, or scattered and discontinuous. Overlapping of receptive fields of receptor units of the different innervating nerves was described. Variation of the adequate stimulus for the elicitation of a response in different receptor units was noted. (Fig. 12) Also it is suggested that threshold for stimulation of receptors in the mandibular nerve was higher than for those of the deep ophthalmic nerve.

The unmyelinated neuronal fiber population supplies information to the brain as to whether tentacle bending is occurring or not. No information is supplied on constant bending states.

Similarly the myelinated neurones propagate information to the brain as to whether tentacle bending is occurring or not. Also they do not supply information as to constant bending states. In addition these myelinated neurones supply information on touch, but only when the pressure exerted by the touching object is changing. There is a differentiation of receptor units here into those which respond when the pressure increases, and those which respond when the pressure decreases.

Thus, in summary the tentacles of Xenopus tadpoles should be thought of as extremely sensitive mechanoreceptor appendages, supplying information to the brain on changing states of touch and bending of the tentacles, but not on unchanging touch and bending of the tentacles. It was armed with this evidence that the investigation into the possible behavioural roles of the tentacle was attempted.

2. THE FUNCTIONAL ROLES OF THE TENTACLES AND THE PHYSIOLOGY OF THE TENTACLE WITHDRAWAL REFLEX.

2. I Introduction.

The functional role or roles of the paired tentacles of Xenopus tadpoles has long been a subject for conjecture. Various workers have suggested functional roles, but it is only in a few instances that they have attempted to verify them experimentally.

Paterson (1939) points out that Bles (1904 - from Paterson, 1939) regards the larval tentacles as being homologous with the balancers of Urodele larvae and that other authorities have compared them with a Siluroid form. Using excision experiments both Paterson (1939) and Brown (1970) found that while the tentacles may serve in maintaining balance to a certain degree, they are not essential to it.

Nikitin (1925 - from Noble, 1931) ruled out the possibility that these larval tentacles had a respiratory function. Similarly Gradwell (1971) maintains that the tentacles play no part in the phasic movements of the mouth which are associated with respiration.

Brown (1970) suggests that the tentacles are used by the tadpoles as "tactile organs for the location of food, especially when browsing near the bottom." This idea seems highly improbable since it would indeed be a very advanced form of sensory discrimination which would enable an animal to distinguish, with purely tactile sensory input, the difference between food and other objects. There is however, the possibility that chemo - sensory elements are present in the tentacle, but this seems improbable since no signs of chemoreceptors were found by either Paterson (1939) or Ovalle (1976).

Gradwell (1971) states that as the turbidity of the water begins to clear the tadpoles increase their depth of swimming and that when all the water has been cleared of food material the

tadpoles can sometimes be observed to flick the tentacles caudally along the bottom and thus cause the organic material in the sub - stratum to be stirred up into the water. Supposedly this is to get food material into suspension so that it can be filtered.

It is probably best to think of filter - feeding in Xenopus in the words of Mc Connell (from Wassersug, 1975), as "trophically analogous to involuntarily filter - feeding zooplankton." Filter - feeding implies non - selective feeding (Wassersug, 1975), or more specifically in the words of Jørgensen (from Wassersug, 1975) as the "uptake of food particles which are too small to be sensed and seen individually." In the light of this it would seem that the observations of Brown (1970) and Gradwell (1971) should be regarded with some scepticism.

It could be possible that Xenopus tadpoles do sense food content in the water, but indirectly. One way this would be possible is via sensory input from stretch receptors in the gut. If Gradwell's (1971) observations of the flicking of the tentacles to stir up the substratum are correct, then it is possible that the drive for this behaviour derives from the 'indirect' sensing mechanism described above. That is, at a critical or threshold level of gut emptiness sensory input, or lack of it, from the gut receptors triggers the abovementioned behaviour.

Another possibility is that the tadpoles use optical cues to place themselves in areas where food concentration in the water is highest. Now, if the tadpoles had an innate behaviour pattern (it would seem unlikely that it could be learnt) which made them move into such areas, driven by light criteria, the initial observ - ations of Gradwell (1971) could be explained, that is the tadpoles increasing swimming depth as the water cleared.

Starrett (1973) has stated that Xenopus tadpoles are able to make slight manoeuvres in their swimming by movements of their tentacles. Any active tentacle movement such as described by Gradwell (1971) and Starrett (1973) would be brought about by

contraction of muscle fibers of the tentacle muscle (Fig. I). The tentacle muscle is a branch of the mandibular levator muscle (Nieuwkoop and Faber, 1967). It segregates out at stage 41 but becomes reduced at stage 61 and loses its individuality at stage 63 (Nieuwkoop and Faber, 1967). It attaches to the base of the cartilage rod of the tentacle on the lateral side (Paterson, 1939). Contraction of the tentacle muscle causes a whip-like caudal flicking of the tentacle (Gradwell, 1971). There is no antagonist muscle to the tentacle muscle and the tentacle returns to its normal position due to the rigidity of the cartilage rod of the tentacle (Gradwell, 1971). In his histological investigation of the tentacle muscle Ovalle (1976) found that two varieties of 'fast' striated muscle fiber and one of 'slow' muscle fibers was present.

Nikitin (1925 - from Noble, 1931)hypothesised that the larval tentacles of Xenopus were tactile in function. Paterson (1939) also mentions the possibility that the tentacles may be tactile although she found no histological evidence for this.

Wager (1965) has suggested that the tentacles stop the tadpoles from approaching the substratum too closely and thereby prevents the tadpoles from gulping up particles as an involuntary consequence of respiratory movements. He does not, however, qualify whether the tentacles serve this function purely mechanically or by tactile sensory feedback.

A role for the tentacles, which has not yet been put forward, is that the tentacles provide the tadpole with information about its anterior environment, of which the tadpole is optically unaware due to the lateral positioning of the eyes.

Recently Cannone and Kelly (1977) have reported on neuro - physiological grounds, that the tentacles are in fact extremely sensitive mechanoreceptive appendages. This study shows that the tentacles enable the tadpoles to detect objects anteriorly to them. Once contact has been established the tadpole has no information as to whether contact persists unless changes in the pressure relat -

ionship between tentacle and object occur.

This study has also shown that the tentacles respond to water currents directed onto them; thus it is possible that the tentacles aid in the orientation response of Xenopus tadpoles to a water current. This orientation response was observed by Shelton (1971) who concluded that the lateral line system could be involved in the response but is not essential to it.

From the above it can be appreciated that the functional role or roles of the tentacles of Xenopus tadpoles is/are some - what in the realm of conjecture with a notable lack of experimental evidence to support the varied hypotheses that have been put forward.

2. 2 Methods and Materials.

Xenopus tadpoles of various stages of development were collected for laboratory experimentation from ponds at the Rose Gardens, Emmarentia, Johannesburg, and a water hazard surrounding the 5th green of the Blue Course of Huddle Park Golf Course, Sandring - ham, Johannesburg. Observations of tadpoles in natural surround - ings were mainly carried out at the various ponds present at the Huddle Park Golf Course.

The tadpoles collected for laboratory work were kept in glass aquaria of various sizes. The 50 l aquaria were usually restricted to holding no more than 100 tadpoles, while another aquarium often used (30 l) was never allowed to hold more than 50 tadpoles. The water in both size tanks was continually aerated.

In newly filled aquaria 'Liquifry' was provided as food material. A few drops of this were added daily until a fairly substantial algal colony had formed in the aquaria. From then on it was found that the water contained sufficient food material for the tadpoles to survive and thus feeding was stopped.

All sensory nerve ablations were carried out under MS-222 anaesthesia. 1 g of MS-222 in 200 ml of water was found to anaesth -

etise the tadpoles within 10 secs. and its effect lasted for up to 2½ to 3 hours. These operations were carried out under saline (Appendix 5) using a Wild M5 dissecting microscope and trans - mitted light. The tadpoles were allowed to recouperate in 5 l perspex aquaria which were found to be ideal for later observations and filming.

Sensory recordings from the optic nerve and muscle recordings from the tail were carried out with 0,04 mm. diameter nichrome wire electrodes, positioned with Prior micro-manipulators. The nerve, supported on the electrode, was covered with a medicinal paraffin/ vaseline mixture (Appendix 6) to prevent dessication.

For the investigations of the tentacle withdrawal reflex polythene suction electrodes, drawn out over a bunsen flame to the required tip diameter, were used for en passant monitoring of sensory input along the deep opthalmic nerve and mandibular nerve, and also for recording electrical activity in the tentacle muscle.

Nerve and muscle activity monitored with these two different types of electrode were first amplified by Grass P5II A.C. preamplifiers and then displayed on a Tektronix 5440 oscilloscope, fitted with a Nihon Kohden Kogyo PC - 2A continuous recording camera for filming purposes. Kodak 35mm. Cineflure film (green sensitive) and Kodak photographic paper were used.

2. 3 Results

2. 3. I The tentacles and balance.

Ablation experiments proved to be one of the valuable methods for ascertaining the likely functional roles of Xenopus tadpole tentacles.

Following removal of the tentacles with a sharp pair of scissors, the tadpoles appeared disorientated when returned to the water. This state could last for up to ten minutes. The amount of time this state persisted for appeared to be directly proportional to

the amount of time the tentacle removal took. This disorientated state during which the tadpoles usually swam around in short bursts, often bumping forceably into objects in the water and the bottom of the tank, also occurred in tadpoles which were similarly removed from the tank and later replaced, but without having their tentacles removed. Thus this aberrant behaviour should be attributed to 'manipulative shock' of the tadpoles rather than some direct resul of tentacle removal.

The tadpoles were seldom observed to be unbalanced after tentacle excision. Mostly the body was still maintained in a plane which appeared the same as in the case of normal tadpoles. In the few cases where the tadpoles appeared unbalanced after tentacle removal they were observed to have a very tilted body plane and seemed unable to maintain a constant depth in the water, often floating to the surface and remaining there, immobile. However, when these tadpoles were observed under the dissecting microscope it was found that in all cases an air bubble had become trapped in the gill apparatus of either one or both sides of the tadpole. Thus the apparent unbalanced state was most probably due to the bubble of air, introduced when the tadpole was removed from the water for tentacle excision, causing buoyancy changes with which the tadpole could neither cope with nor cure.

If one tentacle was removed the plane of the animal's body was never observed to list to one side. Similarly a tadpole never immediately swam in a curve towards the side where the tentacle remained. This type of result should be expected if the tentacles aid in balance.

The behaviour of all these detentacled tadpoles in all subsequent experiments appeared to be exactly the same as for the tadpoles still possessing long, well developed tentacles. There was never any indication that these detentacled tadpoles were less well balanced and hence less adroit in their swimming than normal tadpoles.

Apart from these behavioural observations suggesting that the tentacles play no part in balance, purely physical data also suggests this. The weight of the tentacles in relation to body weight of an average tadpole was always very small (0,0009 g. and 1,2691 g. respectively). In addition, since the tentacles taper towards their tips and thus most of the weight is located towards the base it would appear that such low mass appendages are not physically suited for balancing purposes.

It seems most probable therefore, that the tentacles of Xenopus tadpoles play no part in the balancing of the animal. The hypoth - eses of Paterson (1939) and Brown (1970) should be regarded as misderived from behaviour related to 'manipulative shock' rather than from behaviour resulting directly from the tentacle loss.

2. 3. 2 The tentacles and feeding.

Although Xenopus tadpoles can generally be observed swimming at various depths, fairly often they can be observed apparently 'drifting' along near the bottom with the tentacles in contact with the substratum. The amount of tentacle-substratum contact is variable but generally is quite considerable with about half the tentacles in contact. When doing this the tadpoles have never been observed to gulp up food from the substratum. This would seem to refute Brown's (1970) suggestion that the tentacles are "tactil organs for the location of food."

Furthermore the physical positioning of the tentacles is such that it does not suggest such a function. When the tentacles are in contact with the substratum the tentacles are bent posteriorly, with the result that tentacle-substratum contact is posterior to the mouth opening and also a somewhat large distance laterally from the corners of the mouth on each side. Food material would therefore only be recognised by the tadpoles once the mouth had moved past it. Since the tadpoles have never been observed to move backwards (in fact they are obviously incapable of doing so)



Figure 19. A Xenopus tadpole in its typical free-swimming position. Note the lateral positioning of the eyes causing a blind spot anteriorly.
Calibration; I cm.

this would mean that once food has been located, the tadpole would have to swim through a 360° arc to once again place the mouth in a position to take up the food. Having completed such a complex maneouvre in the absence of sensory cues the question still remains as to how the tadpole is to locate the food material once more, since the tentacles will still be in contact with the substratum lateral to the mouth and the positioning of the eyes makes it certain that a blind spot exists in the anterior midline (Fig. 19). This type of complex behaviour, which would be obvious to an observer, has not been observed during this study. Also such behaviour would be energetically wasteful and, in any event, its very complexity makes it highly unlikely. As a consequence Brown's hypothesis (1970) should be regarded as a most unsatis - factory one.

The findings of Gradwell (1971) that the tentacles were used to stir up organic material from the substratum were also never observed. When the tadpoles 'drifted' along the bottom with their tentacles in contact with it, jerky tentacle movement could sometimes be observed. However, this tentacle movement was never present at the base which would be expected if it were being moved by the tentacle muscle. The tentacle muscle attaches to the cartilage of the tentacle at the base of the tentacle and thus any muscular movement of the tentacle must involve movement of the base. The jerky tentacle movement observed was only present when the tentacles were dragged over a rough substratum, never when they were dragged over the smooth glass bottom of a tank. Therefore, it is likely that the jerky tentacle movement is due to the tentacles being gently snagged on various objects of the substratum. It is suggested that this jerky tentacle movement is what Gradwell (1971) mistakenly identified as caudal flicking of the tentacles.

A further argument against the hypothesis of Gradwell (1971) can be raised. When the tentacle muscle contracts it causes the tentacles to move caudally with a whip-like motion. This, coupled

with the 45° swimming angle of the tadpole, means that if such flicking occurred very little disturbance of the substratum would ensue since the tentacles would effectively be drawn away from the substratum rather than along it. Such disturbances as would occur, would be at the sides of the mouth and thus it would be expected that little material would actually go into suspension in a position where it could be gulped up by the animal. If the tentacles were for this purpose then they could be expected to flick in a dorso - ventral plane so as to stir up organic material directly in front of the mouth rather than behind it.

The tentacles are extremely tenuous and would thus not appear to be very efficient appendages for disturbing the substratum. However, it could be argued that their tenuousness means that they would not stir up too much non-organic material (mud etc.), but rather the much smaller protozoans etc. which the tadpoles feed on.

In fact Xenopus tadpoles were never observed to show directed feeding behaviour. This was the case even when the tadpoles were starved in water which was changed twice a day for a week to ensure that no food material could ever be present, and then Liquifry or a few drops of Paramecium culture were added. The tadpoles were never observed to move into these regions where food material was present even though, with the Liquifry, distinct areas of turbidity were present. Instead the tadpoles continued to 'drift', apparently randomly, around the tank, often into, and then through the very turbid regions rich in Liquifry, without any detectable change in the rate of motion or in the gulping rate. This would tend to support the idea that their filter - feeding is random and apparently not directed as put forward by Mc Connell (from Wassersug, 1975) and Jørgensen (from Wassersug, 1975). This also tends to refute the observations of Gradwell (1971) that Xenopus tadpoles show food-directed behaviour by tending to keep in areas that are turbid. It also refutes the idea of Brown (1970) that the tentacles are "tactile organs for the location of food."

2. 3. 3 The tentacles for manoeuverability.

Tentacle movement attributable to tentacle muscle contraction was very rarely observed in undisturbed tadpoles. Since slight manoeuvres in their swimming were observed fairly frequently without active movement of the tentacles being involved, the statement by Starrett (1973) that Xenopus tadpoles use their tentacles for manoeuverability must remain as unsupported. Furthermore, if Starrett (1973) was correct then there should be a change in the gross manoeuverability of the tadpoles between their early free - swimming stage; their later stages when the tentacles are still developing and even later when they are fully developed. Also, there should be manoeuverability changes after tentacle excission. Such changes were never observed during this study.

During position changes made by the tadpoles, tentacle movement can be observed to occur. This movement, though, can never be seen at the base of the tentacle, but rather is restricted to the distal two-thirds of the tentacle. This movement can therefore be attrib - uted to passive drag of the tentacles, due to their tenuousness. An active tentacle movement due to muscle contraction causes the base of the tentacle to move (Fig. 23) as this is where the tentacle muscle attaches to the cartilage rod of the tentacle.

In cases where the cartilage rod of the tentacle is broken, especially when this is near the base, passive tentacle movement is very much more obvious. This is so since, with the rigidity of the cartilage rod absent, there is less resistance to passive bending. This sort of movement, especially when it occurs distally to a break in the cartilage rod near the base, can very easily be confused with active tentacle movement due to muscular contraction.

In the light of the above observations it is suggested that Starrett (1973) confused cause and effect. Tentacle movement seen during normal swimming manoeuvres of the tadpoles can certainly be ascribed to passive bending. Active , muscle - induced move - ments of the tentacle are rare in undisturbed tadpoles, and a change

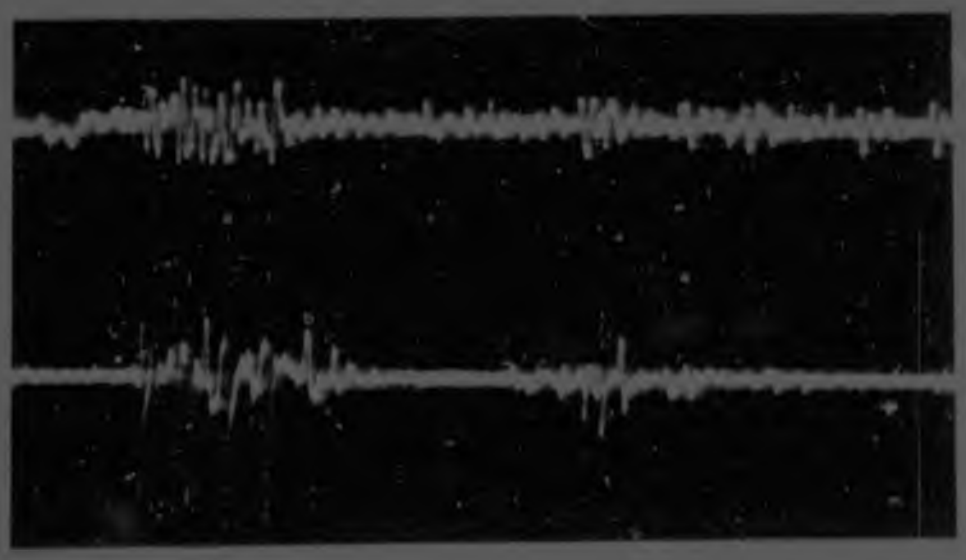

Figure 20. Tentacle withdrawal reflex caused by prodding of the tail of the tadpole. The top trace is a sensory discharge along the deep ophthalmic nerve while the bottom trace is of tentacle muscle activity. Both traces were monitored with en passant polythene suction electrodes. For 'a' and 'b' refer to text.
Calibration; 0,5 sec.

in direction of swimming is not dependent on such movements.

2. 3. 4 The tentacle withdrawal reflex.

2. 3. 4. I The physiology of the tentacle withdrawal reflex.

The only times when active tentacle movement could be regularly observed, apart from extremely rare and apparently spontaneous twitches, was during the tentacle withdrawal reflex. During this withdrawal reflex the tentacle was withdrawn onto the lateral aspects of the head, and often maintained in this position for up to four seconds. In all cases the tentacle withdrawal reflex is associated with rapid swimming movements of varying duration, by the tadpole.

Following the severing of the mandibular nerve proximally to the muscle innervation, the withdrawal of the tentacle cannot be evoked. But if the mandibular nerve is severed distal to the muscle innervation the reflex withdrawal can be evoked. This means that the motor innervation of the tentacle muscle effecting the withdrawal is only present in the mandibular nerve.

The tentacle withdrawal reflex can be evoked via either of the innervating sensory nerves with the other one cut. This shows that supra-threshold sensory input for motor output to cause tentacle muscle contraction can be propagated independently in either sensory nerve innervating the tentacle.

When a tadpole was pinned out live in a dissecting dish the tentacle withdrawal reflex could be elicited in a number of ways. It could be evoked by injurious stimulation of the tentacle, such as cutting, or by prodding of the body surface. Fig. 20 shows a record of a tentacle withdrawal reflex evoked by prodding the tail of a tadpole. In this particular case the tentacle was momentarily rapidly withdrawn caudally. It then slowly returned to its original position, due to the elasticity of the cartilage rod of the tentacle. The tentacle was not actually maintained flattened against the

side of the body as can be observed in some cases, but was rather just jerked away from its anterior position to a caudo-lateral one.

It is suggested that the very short duration and generally larger spikes of the muscle activity trace, such as 'a', are elec - trical activity monitored from the 'fast' muscle fibers mentioned by Oval.e (1976), while the longer duration spikes with lower amplitude, such as 'b', are electrical responses from the 'slow' muscle fibers mentioned by him.

It was never possible to record tentacle muscle activity continuously during a maintained contraction of the tentacle muscle, since this was always associated with violent tail lashing and large amplitude side-to-side movements of the head. In all cases these movements resulted in the loss of the suck of the suction electrode onto the tentacle muscle. It was not possible to completely prevent this tail movement from causing body movement, even by extensive pinning down of the tadpole. This was unfortunate as it meant that one could not investigate whether all muscle fiber types are involved in the maintained withdrawal, or only certain types. From Fig. 20, which is typical of all records obtained, it appears likely that it is mainly 'fast' fibers which cause the initial tentacle movement laterally, and that it is the 'slow' muscle fibers, which only become apparent comparatively later during the muscle contraction recording, that are responsible for maintaining the withdrawal.

Experiments in which the mandibular nerve was electrically stimulated to cause tentacle muscle contraction tend to confirm this. It was found that single supra-threshold 0,02 msec. duration pulses to the mandibular nerve resulted in twitches of the tentacle muscle resulting in rapid caudal flicks of the tentacle. In order to get the tentacle to remain withdrawn repetitive stimulation of the order of 35 to 40 Hz was required. Since no investigation of the time constants of mechanical relaxation of tadpole muscle fibers has been carried out it is not possible to analyse the

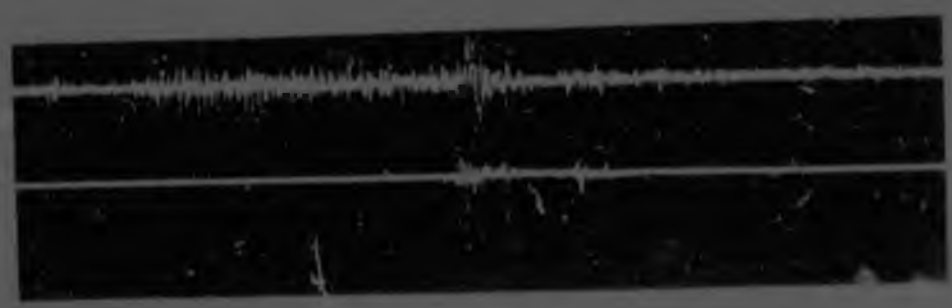

Figure 21. Tentacle withdrawal reflex in response to injurious stimulation of the tentacle. Top trace: sensory activity monitored from the mandibular nerve with the deep ophthalmic cut. Bottom trace: muscle activity. In both cases *en passant* polythene suction electrodes were used.
Calibration; 0,5 secs.

above findings to find which muscle fiber population types cause the maintained withdrawal of the tentacle a such frequencies.

Intracellular recordings with glass pipettes filled with 3 M KCl would have been a means to clarify the above.
However, this proved extremely difficult, probably due to the muscle being very small in diameter, while th rapidity of the contraction of the tentacle muscle always resulted in the electrode leaving the fiber penetrated. Once again it was not possible to adequately stabilise the muscle with pins.

In Fig. 20 it can be seen that sensory discharge from the tentacles occurs fractionally later than the first muscle contract - ion. This can be attributed to a slight delay between the onset of muscle contraction and the beginning of tentacle movement, and hence bending and scraping of the tentacles along the bottom of the dissecting dish

During such tentacle withdrawal due to body stimulation it has been observed that the tentacles can be withdrawn either synchronously or asynchronously. Synchronous withdrawal is the most common, however. This is also the case when tentacle withdrawal is evoked by injurious stimulation of either tentacle.

Fig. 2I shows recordings from the mandibular nerve and tentacle muscle during an ipsilateral reflex evoked by tentacle crushing. Here the delay between the onset of sensory feedback from the tentacle and tentacle muscle activity is quite large. Presumably it is during this delay that sensory feedback reaches threshold for the reflex. However, it should be remembered that the initial portion of the sensory discharge must be due to the forceps used for the crush coming into contact with the tentacle and the resultant tentacle bending causing 'normal' sensory feedback.

Obviously, one would not expect the withdrawal of the tentacle to occur in response to 'normal' sensory feedback from the tentacles. This has been found to be the case and is shown in Fig. 22. In this figure the top trace is of sensory discharge monitored from the

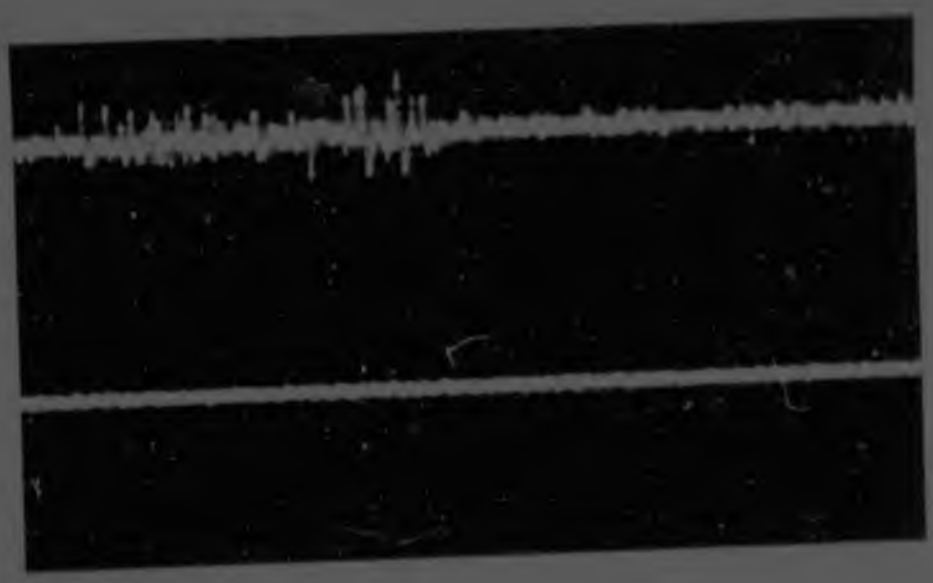

Figure 22. Film record showing that no tentacle muscle response can be monitored in response to 'normal' sensory input. Top trace: sensory discharge monitored from the deep ophthalmic nerve. Bottom trace: tentacle muscle activity. Both monitorings were with en passant polythene suction electrodes.
Calibration; 0,5 secs.

deep ophthalmic nerve with the mandibular cut distally to the motor branch of the tentacle muscle. The bottom trace is of tentacle muscle activity. Here it can be seen that short strokes of the tentacle result in sensory discharge being monitored along the sensory input channel to the brain. However, no muscle electrical activity can be monitored. This indicates that this sort of 'normal' sensory input from the tentacles is below threshold to trigger motor output from the brain to the tentacle muscle.

Following bending of a tentacle, sensory input can also be monitored from the deep ophthalmic or mandibular with the other cut, and once again no motor activity can be observed in response to it.

Careful scrutiny of all discharges from the tentacles leading to muscle contraction has failed to provide any conclusive evidence of the nature of the effective sensory inputs for triggering the reflex. The fact that suction electrodes had to be used meant that the signal to noise ratios obtained were generally low and this made analysis more difficult. However, a number of possible sensory input triggers of the reflex can be considered.

The first possibility is that it is input along the myelinated neurones that triggers the reflex. But this is very unlikely in view of the findings presented in the previous section (Part I) that, using conduction velocity data, sensory responses to stroking and prodding are in myelinated axons. Since it has been shown that 'normal' sensory discharge in response to stroking and bending is not sufficient to trigger the reflex, it would appear that it is not the myelinated neurones that trigger the reflex. Also, since a peak in numbers of axons of a particular size suggests that such fibers are closely associated in function, if myelinated fibers did provide the triggering sensory input then one would expect similar peak diameters in each innervating nerve. This has not been found to be the case (Part I).

A further possibility does exist though, that it is groups of fibers which do not have large numbers of fibers in them that

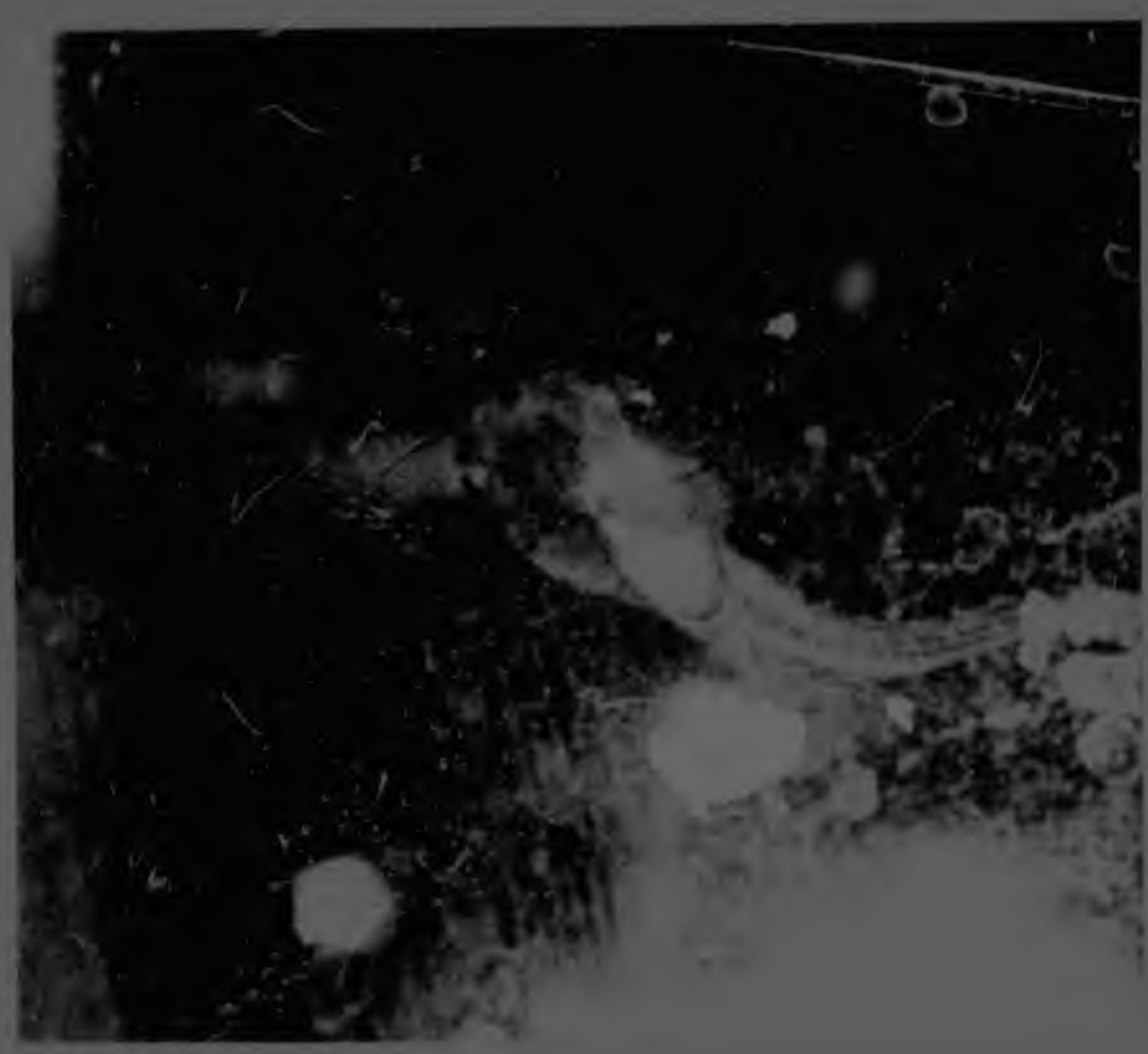

Figure 25. A picture of a Xenopus tadpole during an escape response caused by prodding the tadpole with the glass rod visible on the right of the picture. The involvement of the entire tail musculature can be observed and tentacle withdrawal can be clearly seen.

trigger the reflex. For example axons with diameters of etween 4 - 5μm could be the group since they are present in both nerves and could be the nociceptors described by Maruhashi *et al* (1952). This possibility would be very difficult to test though.

A second possibility is that it is the unmyelinated nerves that propagate the reflex sensory input. This would seem likely since these are the proposed pain fibers of amphibian skin (Adrian, 1925, 1928; Spray, 1976) and also these fibers are common to both of the sensory nerves innervating the tentacle. However, it has been shown in the sensory investigation (Part I) that these unmyelinated fibers can respond to obviously non-noxious stimuli, for example bending. This would make it unlikely that these neurones supply triggering sensory input since it has been shown that 'normal' sensory input does not cause tentacle muscle activity (Fig. 22).

It would appear that the most likely explanation of the triggering sensory input is that the threshold for reflex with - drawal occurs when a large number of axons, both myelinated and unmyelinated, respond. This would not necessarily have to be a high frequency injury discharge since this has been found not to be present (Part I). This appears reasonable since the only apparent difference between 'normal' sensory input (a stroke or bend for example) and triggering sensory input (a crush or cut of the tentacle) is that spike density per unit time is far higher in the latter. It is also possible that it is high density discharge in either the myelinated or unmyelinat J neurones only, that triggers the reflex. This would be difficult to test experimentally.

2. 3. 4. 2 Behaviour associated with the tentacle withdrawal reflex.

The tentacle withdrawal reflex was found to be present during the general escape response of the tadpoles *in vivo* (Fig. 23). During this escape response the tadpoles can be observed to swim rapidly through the water by means of the toil lashing from side-to-

side. In this situation the tadpole uses the entire musculature of the tail as opposed to the normal situation during undisturbed swimming where only flickering of the tail tip occurs.

A functional role for this tentacle withdrawal reflex could be to make the tadpole more streamlined, thereby ensuring unim - peded progress of the tadpole through water vegetation which the tadpoles can often be observed to swim through during the escape response. During the escape response it is apparent that very few sensory cues are acted upon by the tadpole since they can often be observed to swim headlong into solid objects in the water with some force and no apparent decaleration. For this reason the escape response appears to be completely random. Since few sensory cues are apparently used during the escape response, it would seem that having tentacles supplying mechanoreceptive information in front of the tadpole would appear superfluous. In addition it has been noted that the tentacles are extremely fragile appendages which are easily broken along their length or broken off completely. Thus by withdrawing the tentacles onto the lateral aspects of the head the tentacles would effectively be protected from damage.

The reflex withdrawal of the tentacles in response to injurious stimulation of the tentacles themselves can be thought of, in addition to the above, as being the removal of an appendage from a harmful source of stimulation. This is very commonly observed throughout the animal kingdom.

The possession of a tentacle withdrawal reflex, whether elicited via the tentacle itself or via input from other parts of the body, is indicative of a functional role which justifies their main - tainance and protection.

2. 3. 5 <u>The tentacles' role in detecting obj[illegible] in the anterior environment.</u>

The importance of the tentacles to the tadpole in detecting objects anteriorly to them can be illustrated in this example.



Figure 24. A. Spontaneous optic nerve activity.
B. Optic nerve activity in response to a faint shadow moving across the eye. A response con only be monitored when the shadow initially crosses the eye 'a' and when it leaves the eye 'b'. No optic nerve activity is present when constant shadow is moving across the eye (between 'a' and 'b').
Calibration; I sec.

If the optic nerves of a tadpole are severed so that the tadpole then has to rely far more on mechanoreceptive information about its environment, and in addition one tentacle is removed, it is found that a few days after surgery the tadpoles develop a definite bias in their swimming towards the side where the tentacle remains. Since a time delay was present this means that this was not an observation of a balancing effect by the remaining tentacle. This behaviour, apparently learnt, tends to illustrate that the tadpoles appear to be very reliant on their tentacles for information on solid objects ahead and below them.

This importance of the tentacles in detecting objects anteriorly to the tadpoles was thought to be as a result of a blind spot existing anteriorly to the tadpoles. The occurrence of such a blind spot is obvious from considering the lateral positioning of the tadpoles eyes as can be observed in Fig. 19.

Using a nichrome metal electrode to record from the optic nerve it has been found that the tadpole's eyes are very sensitive to faint shadows crossing the eyes. *A sufficient length of optic nerve was cleared, distally to a point of transection, to enable the nerve to be lifted above the saline and wrapped around the recording electrode*

If a shadow is cast onto the eye a sensory response along the optic nerve can be monitored. This response only occurs when the shadow passes over the eye and when it is removed (Fig. 24). Thus the eye apparently only responds to changes in incident illumination upon it and not to constant levels of illumination. Furthermore, no continuous optical sensory input could be recorded, implying that the presence of stationary objects in the tadpoles environment are not being detected. In fact if an object was moved into the visual field and moved around at various rates, no optical responses could be monitored, unless such movements resulted in a shadow being cast onto the eye.

These physiological findings suggest that the tadpole is visually unaware of objects around it, unless the tadpole is moving, or the object is moving and the object casts a shadow over the eye of the tadpole - even a very faint one. This finding emphasises the importance of having tentacles to detect objects in the anterior environment and below the tadpole, since objects in these positions cannot cast shadows onto the eyes and hence cannot be detected visually.

The importance of detecting objects anteriorly to the tadpoles, of which they are optically unaware, was unwittingly pointed out by Wager (1965) when he mentioned that the tentacles may serve to prevent the tadpoles from gulping up the substratum when near the bottom. However, the possibility exists that it is not merely sensory input which prevents this happening, but rather also, or only, due to the rigidity of the tentacle mechanically preventing the tadpole from approaching the bottom too closely. This proposed mechanical action would seem important since the tadpoles have no backward form of locomotion. Thus the tentacles could act as a buffer when a tadpole drifts gently into a solid object.

To test the mechanical properties of the tentacles, a number of tadpoles were operated on, and their mandibular nerves (distal to the mandibular muscle innervation to enable respiratory movements to continue)and deep opthalmic nerves cut as close to the entry point of the tentacle as possible. This deprived the tadpoles of sensory inputs from the tentacles. Behavioural observations could now hopefully provide information on a possible distinction between a sensory function and a purely mechanical function of the tentacles.

With such tadpoles most of their behaviour remained identical. Free-swimming position, balance, manoeuvrability and escape response were not observably affected, except that now no escape response occurred following injurious stimulation of the tentacles. One difference present was that when tadpoles drifted into a solid object, such as the side of an aquarium or some plant material,

considerably more tentacle bending occurred before the tadpole became stationary. In the cases of tadpoles with all nerves intact, they could often be observed to drift into an object and become stationary with only about one-quarter to one-third of the tentacles flattened against the object. This was not observed in cases of tadpoles with denervated tentacles. Here tentacle contact with the solid object was usually about two-thirds or more along the length of the tentacle before the tadpole became stationary.

These observations imply that the drifting of the tadpoles is due to some forward component of the tail flickering which can be inhibited following sensory input from the tentacles. Once this motor output to the tail has ceased, the mechanical properties of the tentacle stop further forward movement. In the cases of the denervated tadpoles, sensory feedback from the tentacles could not occur and thus a greater contribution from the mechanical properties of the tentacle was required before the momentum of the body, and the forward component of the tail flickering could be overcome and the forward movement stopped; hence greater tentacle-object contact.

Thus it would appeur likely that the tentacles serve two functions when a tadpole comes into contact with a solid object. Firstly, the sensory feedback from the tentacles inhibits that component of tail flickering which causes forward 'drifting', and secondly that the tentacle has mechanical rigidity which stops forward movement. The importance of the above mechanisms involving the tentacles in preventing a tadpole's mouth from coming into contact with objects in the water is indicated by the following observations.

When long filamentous algae (for example Cladophora) was well established in aquaria where tadpoles were present, it was found, in many cases, that tadpoles became trapped, the algae being gulped into the mouth and becoming trapped in the gills. This was noted most frequently in tadpoles without tentacles, including pre-tentacle stage tadpoles. Tadpoles with well-developed

tentacles were also trapped, albeit much less frequently.

Further observations reinforce the hypothesis of the tentacles being used to probe the anterior environment of the tadpole.

Xenopus tadpoles are generally found in ponds of water having fairly abundant vegetation. The most common vegetation associated with these ponds is various water weed (Lagarosiphon, Elodea, etc.), grasses (Pycreus, etc.) and various reeds. Usually tadpoles main - tain themselves in clear water away from such vegetation. In one articular large pool of water, the water weed Lagorosiphon was well established and had colonised the whole pool. The only open water present was in small isolated patches within the weed which were up to half a meter in diameter. Xenopus tadpoles were nearly always observed near the centre of free-water and only rarely near the weed surrounding it. It was even more rarely observed that the tadpoles were in tentacle or bodily contact with the weed. When the tadpoles drifted towards the weed they could be observed to turn and swim away from it in most cases. This was apparently due to visual cues since contact with the weed was rarely established.

If the tadpoles, in such an open patch of water, were startled, for example by throwing a stone into the water, the escape response was elicited. Often this resulted in the tadpoles actually swimming into the water weed. In many instances it was noted that many of the tadpoles which swam into the weed became temporarily trapped in it and only escaped from it by violent tail thrashing behaviour. In some cases this resulted in the tadpole moving further into the water weed, thereby adding to its plight. Similar situations resulted when the tadpoles were observed to escape into reeds and grass growing in the water near the edges of ponds where tadpoles were present.

These observations suggest that it is important for Xenopus tadpoles to keep clear of thick vegetation in the water during their everyday lives. This is so because their apparent low manoeuverability and low mass prevents them from efficiently

extracting themselves from vegetation once they have moved into it. Also the violent activity associated with attempts to extract themselves from the vegetation would seem to be disadvantageous since predators could be attracted.

Neurophysiological recording of responses from the optic nerve (page 54)has provided an indication of the effective stimuli that are probably responsible for the normal avoidance behaviour which the tadpole displays towards vegetation in its normal environment. With such optical feedback tadpoles could conceiveably maintain themselves in open water during daylight without further sensory cues. This is because all vegetation must throw some shadow into the water, except close to the surface where Xenopus tadpoles are seldom present anyway. Thus if the tadpoles always moved into the lighter side of a light-dark interface they could maintain themselves in open water where no vegetation is present.

This suggested behavioural mechanism for avoiding vegetation in the water is all very well when light is present, but during very dark nights, especially if the water is turbid, this optically - dependent behavioural mechanism may not be sufficient. It is hypothesised that the tentacles substitute for the eyes in vegetation avoidance behaviour when such environmental conditions prevail.

To test this the optic nerves of ten tadpoles of similar stages (56/57) were surgically cut and in addition, the tentacles of five were removed. The tadpoles were then placed in a large aquarium (50 l.) in which Lagorosiphon had been placed around the edges leaving a patch of open water in the middle. The tadpoles were observed as the anaesthetic wore off and extracted from the Lagorosiphon when they swam into it in their disorientated state. Once all the tadpoles had recovered fully from the anaesthetic it was observed that none of them could maintain themselves in the middle of the open water patch. A control group of tadpoles with optic nerves and tentacles intact, succeeded in maintaining themselves in the open water, suggesting that it is visual cues that

maintain the tadpoles away from surrounding vegetation.

All the optically denervated tadpoles were observed to 'drift' into the water weed fairly frequently. When this happened to those with tentacles, following initial tentacle contact of the water weed, forward movement ceased and then the tadpoles either remained stationary for a while or flicked themselves around onto a roughly perpendicular course and 'drifted' off. If this once again resulted in head-on contact with the vegetation then a similar event occurred In all cases it appeared the tadpoles had sufficient distance be - tween themselves and the point of tentacle contact with the weed, for manoeuverability.

However, with the detentacled tadpoles it was found that when they came into physical contact with the water weed it was with the mouth regions. Under these circumstances manoeuverability was reduced.

During one 12 hour period, during which checks were made every hour, it was found on four occasions that detentacled tadpoles had become hopelessly entangled in the water weed. It was only on one occasion that a tentacled tadpole was found to be hopelessly entangled. Similar disproportions were noted when this same tank was periodically checked during succeeding days.

The mechanical 'braking' effect of the tentacles on contact with vegetation, coupled with the apparently inhibitory effect of tentacle sensory feedback on the 'drifting' type of locomotion, together result in the maintainance of a space between the tadpole and the material with which it comes into contact such that the potential for adequate manoeuverability is retained.

2. 3. 6 <u>The tentacles in an orientation response.</u>

In the light of the finding of Cannone and Kelly (1977) that a sensory discharge can be monitored from the tentacles following the application of water currents onto them, and bearing in mind that Shelton (1971) found that the lateral line could be involved

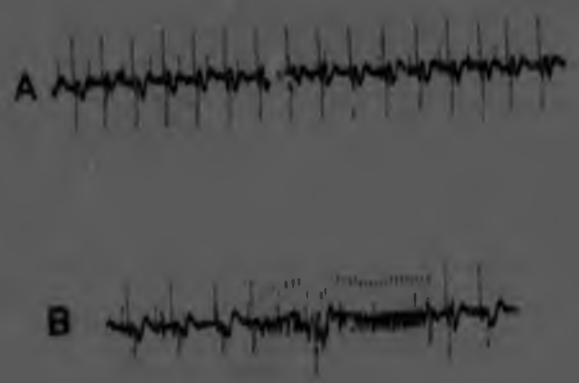

Figure 25. Tail muscle responses monitored with a nichrome wire electrode. A - extracellular muscle action potentials monitored from muscle fibers responsible for tail flickering. B - muscle recording of the tail showing the high frequency contractions of muscle fibers within the tail during an escape response.
Calibration; 1 sec.

in the water current orientation response observed in Xenopus tadpoles, but was not essential to it, it is possible that the tentacles play a part in this orientation response. This was tested both behaviourally and physiologically.

When a metal electrode was inserted at different levels into the tail of a live tadpole pinned down onto a dissecting dish, the muscle contractions of the tail causing flickering of the tip could be monitored (Fig. 25A). The dissecting dish which was used in such experiments was especially modified so that the saline in which the tentacles lay was effectively isolated from that bathing the rest of the body. This was done by means of a horizontal piece of perspex which extended from one side of the dissecting dish to the other. A rectangular piece was cut out from the middle of the perspex so that the anterior regions of the tadpole's head could extend through it, from one compartment of the dissecting dish into the other. Once the tadpole had been pinned out in this position medicinal vaseline was smeared over the projecting parts, excluding the tentacles, to block the anterior lateral line system and to seal the spaces left between the tadpoles head and perspex. In this way water currents could be applied to the tentacles without stimulating the lateral line system, while recording muscular activity of the tail.

The results of these experiments were unambiguous. Water currents applied selectively to the tentacles did not elicit any change in the frequency of tail flickering. However, if one of the tentacles was crushed, comparatively high frequency tail muscle contractions could be monitored (Fig. 25B), supposedly part of the escape response.

Since this sort of physiological evidence should be regarded with scepticism due to the severe experimental manipulation of the tadpoles that was necessitated, confirmation was sought at the behavioural level.

When water currents were applied through small diameter

pipettes onto the bodies of tadpoles, orientation responses were often elicited confirming the earlier findings of Shelton (1971). But when the water current was restricted to the tentacles no such orientation response was ever observed. Moreover, pre-tentacle stage tadpoles and those with tentacles removed can also be observed to respond to a water current by orientating themselves into it, and appear to do it as well as tadpoles with tentacles.

These observations support the physiological experiments in that the tentacles do not seem to contribute to the orientation response of the tadpoles to water currents, this response seem - ingly being due to sensory feedback from the lateral line system (Shelton 1971) and other factors which are so far unknown but may be visual

2. 4 Conclusions.

At the outset it should be stated that without the neurophys - iological results this behavioural investiagtion would have been far more difficult than it proved to be. Muntz (1971) has pointed out that comparatively few attempts have been made to use phys - iological findings to explain behaviour. In this investigation where physiological findings have been used to ascertain the function of a body appendage, the importance and usefulness of this type of approach has been emphasised. It is clearly of great importance to know the characteristics of an animal's sensory mechanisms in terms of effective stimuli, before behavioural roles can be understood.

The lack of knowledge of an animal's sensory mechanisms often leads to intuitively possible, but factually misplaced conclusions. Such cases have been encountered in the literature relating to this investigation (Brown, 1970, Gradwell, 1971).

The physiological investigations greatly reduced the possible functional roles of the tentacles, and meant that the behavioural investigation could be channeled into specific directions.

It would appear that the one main function of the tentacles is the placing of a mechanoreceptive area of skin in a position which is important to the survival of the tadpoles. From these investigations it appears very likely that by having such anteriorly projecting appendages possessing physical rigidity, the tadpoles are able to overcome their inherent lack of manoeuver-ability, stemming from their having only one propulsive means, the tail. This would appear to be especially important in situations where optical information is considerably reduced due to water turbidity or lack of light.

Moreover, the lateral position of the eyes with their limited frontal field of view, coupled with the fact that visual sensory feedback occurs only in response to moving shadows, suggests that visual cues cannot be relied upon alone to provide the tadpole with adequate sensory information regarding features in its anterior environment. Possession of mechanoreceptive appendages projecting anteriorly would therefore seem advantageous to the tadpole in its natural habitat.

Towards the end of metamorphosis the eyes migrate to a more antero-lateral position altering the visual field to a more anterior one (Brown, 1970). It is at this stage that tentacle degeneration occurs and this lends further credence to the suggestion that the tentacles complement visual sensory information. It is also during these late stages of metamorphosis that the hind limbs of the tadpoles become functional and aid the tadpole's manoeuverability (Hughes and Prestige, 1971). This makes the tadpole far more manoeuverable, and thus mechanoreceptive appendages, functioning to assure the maintainance of the tadpole in a manoeuverable position, would seem superfluous.

Although this investigation has pointed to the functional role

of the tentacles of Xenopus tadpoles, the question as to why the tentacles appear only fairly late in development remains unanswered. Clearly a more ecological approach is needed to solve the problem.

Appendix 1.

Formaldehyde/Gluteraldehyde fixative.

Paraformaldehyde	1,2 g.
Water	20,0 ml.
Cacodylate buffer (Appendix 2)	10,0 ml.
Gluteraldehyde (25%)	5,0 ml.
Calcium solution (see below)	0,4 ml.

The water was heated but kept below 60° C and two drops of I N NaOH were added. The paraformuldehyde was then dissolved in the water and the mixture allowed to cool. Once cool the other components were added. The calcium solution consisted of I g of $CaCl_2 6H_2O$ (hydrated calcium chloride) in 100 g of water.

Appendix 2.

Cacodylate buffer.

Sodium cacodylate	42,8 g.
Hydrochloric acid (I N)	6,9 ml.
Water to 1000 ml.	

The above components were mixed without heating.

Appendix 3.

Osmium tetroxide fixative.

Osmium tetroxide	I g.
Cacodylate buffer (Appendix 2)	40 ml.
Water	60 ml.
Calcium solution (Appendix I)	I ml.

The above components were mixed in a fume cupboard.

Appendix 4.

Araldite embedding medium.

Araldite cy 212	10 ml.
Dodecenyl Succinic Anhydride (D.D.S.A.)	10 ml.
Di-Butyl Pthalate (D.B.T.)	1 ml.
2,4 dimethylaminoethyl phenol (D.M.P.-30)	0,3 ml.

The above were measured out gravimetrically into a 30 ml gloss bottle. The bottle was then stoppered and the mixture rotated overnight at room temperature.

Appendix 5.

Saline.

NaCl	65,0 g.
KCl	4,2 g.
$CaCl_2 2H_2O$	2,5 g.

The above ingredients were dissolved in 10 litres of water in the order that they are presented above.

Appendix 6.

20 ml of commercial vaseline was heated until in a fluid state and was then added to 30 ml. of medicinal paraffin with continual gentle heating and stirring of the mixture. This resulted in a mixture which was just fluid enough to drip from a glass rod which was submerged in the mixture and then removed. This was found to be the ideal consistency for the mixture.

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Author Kelly P J
Name of thesis A Neuroethological Investigation of the Pasred Tentaccles of Xenopus Tadpoles 1978

PUBLISHER:
University of the Witwatersrand, Johannesburg
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