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CEPHALOPODS AND NEUROSCIENCE

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INTRODUCTION

Study of cephalopods at marine laboratories has provided material for some of the outstanding discoveries of neuroscience in this century. The giant nerve fibers are the most conspicuous example, but studies of photoreceptors and the memory mechanisms of the brain have been very fruitful, as has work on chromatophores and many other topics. It would be impossible to summarize all this work but it may be interesting to show the sequence in which some of it has developed at Naples, Plymouth, and Woods Hole, in much of which I have been concerned.

EYES

Perhaps the earliest contribution of cephalopods to fundamental neural processes was the discovery of the electroretinogram by Fröhlich at Naples in 1914. The curious electrical phenomena in the rhabdomes are still only partly understood and have been the subject of many later investigations. Outstanding has been the demonstration by Hagins and McGaughty (see Messenger, 1981) that the opening of channels to produce generator potentials takes place locally, near the site of photon absorbtion in a rhabdome. Speaking of the retinal pigments will remind us of the use made by Hubbard and Wald at Woods Hole of cephalopod eyes to provide the rhadopsin for their fundamental research. Indeed the history of the use of these eyes for neuroscience merits a symposium of its own. Cephalopods appear to have no color vision, in spite of their own colorful displays (Messenger, 1981). But the capacity to detect the plane of reflected polarized light, first suggested by the geometry of the rhabdomes (Young, 1960; Saibil, 1982) and proved experimentally at Naples by Moody and Parris (1961) and by electrical recordings, may provide a sort of substitute for color vision (Saidel *et al.*, 1983).

STATOCYSTS

The statocyst is an organ, one of whose major functions is stabilization of the visual image. It has been investigated at Naples by Young (1960) and Wells (1960). Thorough studies have been made by Budelmann at Regensburg using large numbers of octopuses and cuttlefishes carried alive from Naples. He and I have analyzed the oculomotor control system (Budelmann and Young, 1985). Recently he has discovered that an octopus monitors its fast and slow movements separately by a unique system of large and small cupulae (Budelmann and Williamson, 1985).

Another recent development has been the discovery that the peduncle lobe and basal lobes of the brain contain systems of small cells with parallel fibers. These resemble the vertebrate cerebellum and like that organ are involved in the optomotor reflexes.

Measurement of the statocysts of many species collected from Plymouth, Naples, Woods Hole, Miami, and Hawaii have shown a system similar to the semicircular canals of vertebrates (Stephens and Young, 1975; Maddock and Young, 1984). The

canals are formed by a series of knobs, the anticristse. They are best developed in the rapidly moving loliginids and ommastrephids. In the slowly moving neutrally buoyant forms, the statocyst is large and empty.

Extraocular Photoreceptors

What may be called the modern epoque of cephalopod research at Naples was begun by Enrico Sereni who made many experiments on the chromatophores and salivary secretion, summarized in a long article in 1930. At the time of his early death he and I were collaborating in a study of regeneration of the stellar nerves, which was completed after his death (Sereni and Young, 1932). In the course of this study I was attracted to a small orange spot at the hind end of the stellate ganglion of *Eledone.* I cut sections of it, with no hypothesis other than curiosity. It proved to be a hollow vesicle into which passed a number of projections, apparently of nerve cells. After discussion with Sereni it was named the epistellar body (Young, 1929). I was interested at the time in the vertebrate adrenals and made the hypothesis that these projections into the epistellar cavity were secretory. Ernst Scharrer eagerly seized upon this as one of the earliest examples of neurosecretion. But he and I were sadly mistaken. Forty years later Howard Bern, himself an endocrinologist, thought it time to study this organ properly. The E.M. quickly showed that the processes inside the epistellar body are *rhabdomes* (Nishioka *et al.,* 1966). It is not a gland at all but a photoreceptor, though without any lens or other dioptric apparatus. Alex Mauro working at Naples and Ischia confirmed that it produces its own minielectroretinogram (Mauro and Baumann, 1968). What can this photoreceptor be doing *inside* the mantle? The epistellar body is especially large in deep-sea octopods, which are transparent. One hypothesis is that it serves to detect the presence of a mass of luminous material in the mantle, which would attract a predator. The oesophagus is deeply pigmented, presumably for the same reason. However Houck (1982), working at Hawaii, has recently shown that in octopuses with the optic nerves cut diurnal rhythms can still be entrained by light, perhaps detected by the epistellar bodies.

The extra-ocular photoreceptors in decapods are in the head, not on the stellate ganglion (Thore, 1939; Boycott and Young, 1956). They have been thoroughly studied by R. E. Young (1978) at Hawaii in many species of squid. In some mesopelagic forms such as *Abraliopsis* they serve to monitor the downward illumination emitted by photophores for countershading. For this light to be effective in making the squid invisible from below it must match the downwelling light. This match is ensured by the photosensitive vesicles which are in two sets, one looking up to the surface and the other towards the animal's own luminous organs (Young and Roper, 1976). The system even ensures an appropriate match to the wave length, if necessary, in moonlight!

The extra-ocular photoreceptors are even larger in the bathypelagic squids, such as the cranchiids, many of which proceed to depths beyond the range of daylight, especially for reproduction. Here the photoreceptors must have another function. I suggest that they monitor the depth at which to spawn. They provide huge irregular masses of photosensitive material and their nerves connect with the peduncle lobe of the brain, which is probably concerned with movement in the vertical plane. It may be that the squids continue to proceed deeper and deeper until no photons are captured even by these large masses of pigment. When the light goes out it is dark enough to breed! Conversely the photoreceptors prevent rising into the dangerous lighted zone.

GIANT FIBERS

These discoveries are all exciting but even greater developments have flowed from my original curiosity about the epistellar body. Having found it in octopods I naturally also made sections of the stellate ganglion of decapods. No epistellar body was there but instead I found the giant nerve fibers. I am often asked at what date this discovery was made but can give no clear answer. Sections of the ganglia of *Loligo* were made at Naples in 1929 but at first I thought these large spaces were veins. The axoplasm does indeed look quite like blood in some sections. Then I followed them towards the hind end of the ganglion where, as we now know, they originate by the fusion of the axons of many cells. This seemed to me, as a faithful Oxford follower of Sherrington, to be so unlikely that it took some years to persuade myself of it. However in the collection there is a slide, labeled in my handwriting "1 · 5 · 30," which is a thick section clearly showing the axons dividing and passing to many cells of the giant fiber lobe. So I must have "known" their anatomy at that date, but was not sure enough to publish.

During the early 1930s I worked at Plymouth, mostly with *Sepia,* where the giant fibers are smaller and there is no giant fiber lobe. The first publication was therefore a note in the *Journal of Physiology* in 1934 claiming that the axoplasm is fluid, which we now know to be an error. Then in 1936 there was a fuller account suggesting that the epistellar body had been derived from the giant fiber lobe of an ancestor, which is probably another error.

However by now I was fully convinced that they were nerve fibers, and in 1936 was able to prove this by simple experiments at Woods Hole (Young, 1938). Several others then joined me: Bronk, Gerard, and Hartline all tried to show action potentials but the primitive oscilloscopes of those days worked poorly and my distinguished colleagues could not show reliable action potentials by electrical stimulation. One day Keffer Hartline and I hooked a fiber to an amplifier and speaker and put a crystal of oxalate on the end; out came a wonderful buzz—the first giant fiber impulses.

K. C. Cole and Curtis were soon studying the electrical properties of the membrane and Frank Schmitt and Richard Bear showed me how to study biophysical structure properly (Bear *et al.*, 1939). Material collected that summer at Woods Hole provided the basis for a full study of the giant fiber system of *Loligo* (Young, 1939). It was only at this time that I discovered that the first order giant cells in the brain had been illustrated by Williams (1909). His excellent monograph was published in Holland and so far as I can discover the giant fiber system was never mentioned throughout the succeeding years. Williams followed the large fibers into the stellate ganglion and stellar nerves but he seems to have supposed that they ran through the ganglion without synapse. He gave no figure of them.

The next phase of work on the giant fibers was mainly at Plymouth. Pumphrey and I showed (1938) that the conduction velocity follows the square root of the diameter. Rapid conduction by giant fibers is an expensive luxury for a species. In these experiments we were helped by Alan Hodgkin, then a student at Cambridge; this was the first introduction of the Cambridge team to the squid fibers.

The axons provided the material for the first direct measurements of the internal potassium concentration of protoplasm, made independently by Bear et al. (1939) and Webb and Young (1940).

The full development of the potentialities of the giant fibers occurred after the war and I shall not try to follow the details. Outstanding achievements were the placing of an internal electrode and the emptying and refilling of the axon by

Hodgkin and Huxley. These investigations provided the data that enabled them to deduce the equations of the ionic exchanges that are involved (Hodgkin and Huxley, 1952).

The special usefulness of the fibers is that they allow monitoring by electrodes on both sides of the membrane. Numerous workers have used this property for studies of membrane transport at Woods Hole, Plymouth, and Naples, continuing to the present day at Plymouth with the work of Keynes and Baker and Haydon, to mention only three out of many. Miledi and Katz and other groups have been able to study the two sides of the synapse at Naples. The masses of axoplasm and sheets of membrane have provided opportunities for the work of thousands of physiologists, biochemists, and biophysicists and will continue to do so in the future. As new problems and techniques appear these fibers will provide the material of choice for testing them. It is curious to think how different neuroscience would have been had I not made sections of a yellow spot—*out of simple curiosity.* It is easy to say that the fibers would have been discovered by someone else soon. But would they in the present climate? Who would write a grant application to study the possible structure of an unknown organ? It is an example of the need to allow people to pursue whatever curious subject may interest them.

Memory

The sections of the brains of squids and cuttlefishes that were made to study the giant fibers showed me many other wonderful things. The supraoesophageal lobes include a dozen distinct lobules, each with a different pattern of cells and neuropil. Surely these would provide a good opportunity to study higher nervous activities, such as memory. I felt that this was an opening even more important than was offered by the nerve fibers. Already in the 1930s there was a moderately good idea of how nerve impulses are conducted. Hodgkin and Huxley were able to carry this much further and the giant synapse provided great opportunities. But the really mysterious problems of neuroscience were hidden there in the neuropils of the higher centers. Biophysics was not ready to attack them, and still cannot do so even in 1984.

However it seemed to me that a start should be made, and Sanders and I were able to show at Plymouth that the learning power of *Sepia* is indeed dependent on the vertical lobe (1940). After a long interval in the war, while studying nerve regeneration in mammals and men, I returned to the problem of memory at Naples. *Octopus* provides even better opportunities than *Sepia* and proved to be a splendid learner. The supply at Naples seems to be inexhaustable. The Posillipo fishermen have been able to bring in 20 or more octopuses a day in excellent condition and the Stazione has generously provided space for special tanks to be built with funds from the British Science Research Council. These facilities are still available.

With the cooperation of Boycott, Wells, Sutherland, and many others, the two memory systems of the octopus, visual and tactile, have been thoroughly explored (see Wells, 1966; Young, 1983). Lesions have shown that various lobes are involved in learning, each in a different way. The visual and tactile memory systems each includes four lobes with distinctive structure and function. Unfortunately for some reason it is difficult to record the electrical activities of octopus neurons. The afferent fibers proceeding from the retina and statocyst have been thoroughly investigated, but little is known about activities within the brain. There have been many investigations of the transmitters involved since the classical demonstration by Bacq (1937) of the huge amounts of acetylcholine in the optic lobes. Among

many others Juorio (1971), Juorio and Barlow (1976), and Tansey (1979) have shown the distribution of amines in the brain, mostly using material obtained at Naples.

Studies of cephalopods have not revealed all the secrets of the mechanism of learning but they have shown much, and may show more. It may be claimed that we already know from work with large-celled gastropods, such as that of Kandel, that memory involves changes in synaptic conduction. This is a great advance but does not tell us how representations stored in the brain enable an animal or man to recognize a rectangle. There are properties of *aggregates* of neurons and we still require brains such as those of octopuses that are suitable for studies of them. It will need special methods that cannot yet be seen, and I doubt whether multiple electrodes will serve. Some methods must be devised that can show how numerous neurons interact. The various neuropils of an octopus may provide the material that is needed, just as the giant fibers of the squid will allow testing of new methods for the study of membranes.

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