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RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

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RESEARCH WORK

Introduction

Five principal modes of study are available for the analysis of behaviour: (1) morphology of the nerve cells which control behaviour directly, (2) the activity of the same nervous pathways of excitation, (3) analysis of the controlled accumulation, localization and release of secretions which are active controlling agents, (4) observation in the field or in the laboratory of the full range of normal behaviour, (5) comparison with theoretical models. All these aspects have to be studied if the mechanism of a behaviour pattern is to be understood. No one aspect can explain any other aspect, but all these types of activity lean upon each other and contribute to an understanding of how and why animals act as they do.

Most of the analysis of behaviour cannot be successfully carried out because the processes of interest are inaccessible in very small or complex systems, but certain preparations, drawn from a variety of lower animals, especially insects, molluscs, etc., lend themselves conveniently to an elucidation of the principles by which all animals are thought to work. Much detailed anatomy, and many microelectrode studies are required before even a small part of an animal's actions can be analysed mechanistically.

A practical way to achieve any results in detail is to limit the study to one or two amenable systems in a few available animals, chosen to be approachable by several methods bearing upon the one problem. This is the reason that most of this group work on the process of visual perception by the compound eye.

The compound eye of insects consists of thousands of facets, behind each of which there lies a group of nerve cells specialized as light receptors. Each receptor has a nerve fibre which runs back to an integrating ganglion behind the eye. There is a succession of such ganglia and the information received by the numerous receptors in parallel is processed in such a way that only relevant aspects of it are important in the resulting visual responses of the animal. Data processing of this kind is a necessary part of perception. In the compound eye of favourable large insects it is possible to analyse the process of light capture by single cells, the integration in successive ganglia, the overall perception by the whole animal and the growth process by which the complex patterns arise in the first place.

The process of analysis, from receptor through the central nervous system to a study of whole animal behaviour is not restricted to the eye. Dr. Young is studying selected insect mechanoreceptors and Mr. Shelton is working on underwater vibration receptors of the tadpole lateral line with the same objects in view.

Movement of Crab Eyes

(G.A. Horridge)

Observation of eye movements is a useful technique in determining what an animal looks at. Numerous studies on the eye movements of restrained crabs have shown for example that shore crabs can see the movement of the stars on a clear night. The present work is an analysis of eye movements in freely moving crabs to see whether the eyes fixate upon surrounding objects when the crab is walking sideways and when it turns round. In general the results are closely comparable with the behaviour of man in the same situation.

Movement of Retinula cells in insect eyes on light adaptation

(Walcott and Horridge)

The sensitivity of insect compound eyes to light varies with the previous illumination of the animal. A dark adapted eye has a lower threshold to light than the light adapted eye. Despite models which suggest a simple cause of this change, several factors are clearly at work. A rapid change in sensitivity occurs in the primary receptors themselves: slower changes in sensitivity have been ascribed to pigment movements, and changes in field size to movement of mitochondria and endoplasmic reticular spaces. In the course of studies on the physiology of the retina of *Dytiscus marginalis*, we have discovered that light and dark adaptation is accompanied by large movements of the retinula cells. Examination of the retina of the giant water bug, *Lethocerus americanus*, has revealed a similar phenomenon. We are presently examining a number of species of insects to determine the distribution of the phenomenon. We are also investigating physiologically the changes that occur on light adaptation.

Length-tension relations in an insect muscle

(Walcott)

I have found an insect muscle which is able to develop significant tension over extreme degrees of stretch (up to 175% of rest length). Since such behaviour is inconsistent with current models of muscular contraction a close examination of the structure of the insect muscle is being made with both the light and electron microscope. Such a study is revealing differences in the structure of this muscle from other muscle which could account for the differences in physiology.

The Structure and physiology of the retina of Lethocerus
(A giant aquatic insect)

(Walcott)

A study of the structure and physiology of the retina of Lethocerus, a giant water bug, is in progress. This study is part of a large programme involving a correlated anatomical and physiological study of a number of insect retinas.

The structure of the eye of a Megalopteran insect

(Walcott and Horridge)

The megalopterans are a primitive group of insects. It is therefore of interest to see how the structure of their retinas differ from more recently evolved species. The retina is peculiar in that there is a large clear space, traversed by no obvious light thread, between the cones and the receptor area. This feature is again found in the gyrinid beetle and it is at present impossible to understand how such eyes can see detailed objects when dark adapted.

Structure and function of the brain of brachyceran crustaceans

(D.C. Sandeman)

All my investigations are basically related to brain function in brachyceran crustaceans. A local species of shore crab, Leptograpsus, is proving to be a useful experimental animal. It lives amongst the rocks along the N.S.W. coast, is fairly easy to catch and survives well in open tanks in the laboratory in Canberra.

Conventional histological methods show that the main nerve tracts and neuropile complexes in the brain and optic lobes are very similar to those described in Carcinus, a crab which has been the subject of previous research (Sandeman 1969). Electrophysiological tests to show the relation of the various neuropile areas to one another are still being set up.

A preliminary experiment on the regeneration of motor axons to the eye of Leptograpsus has also shown some promise. Crushing the eye nerves in this crab causes complete dysfunction of the eye stalk for about four days after which eye movements slowly recover. Complete recovery of optokinetic mystagmus takes about eight days. Clearly a careful check on the damage produced by nerve crushing must be carried out. If it is found that this method actually causes the axons to lose continuity, the implication follows that the regenerating motor nerves connect correctly to the various eye muscles. Leptograpsus is peculiarly suitable for this kind of experiment because of its ability to live out of water and at a fairly high temperature. Regeneration in other arthropods (i.e. cockroaches) has been shown to be faster at higher temperatures.

Another local crab Heloecious has been used to advantage to investigate the function of the medulla terminalis, a neuropile area situated in the optic lobe. This neuropile has been shown by Maynard and Yager to be intimately concerned with olfaction and the input from the antennules. However it was not known if the input from the statocysts at the base of the antennules also passed the medulla terminalis. Heloecious has characteristically long eye stalks so that the optic lobes, including the medulla terminalis can be removed without destroying the muscles which move the eye stalk. Animals treated in this way still have geotactic eye responses showing that the control of this system does not need the optic lobes or medulla terminalis.

Insect mechanosensory systems

(D. Young)

The fine structure and electrophysiology of insect mechanoreceptor systems is being studied under three main headings.

1. The properties of individual sensory interneurons in the cockroach (Periplaneta americana) and the fly (Calliphora viccinia).
2. The patterns of growth and regeneration of the above interneurons.
3. Incidental studies on the auditory receptors and interneurons of convenient Australian species of insects.

Changing role of a sense organ system during development

(P.M.J. Shelton)

Just how sense organs of developing animals are adapted to the concurrent changes of behaviour is unknown. A study of the anatomy and physiology of the lateral line system of Xenopus laevis has shown that there are considerable changes of structure (and presumably function) of the peripheral lateralis system during development. The numbers, distribution and orientation of organs is seen to change at metamorphosis and must result in a different sensory input to a given stimulus in tadpole and adult. Present studies show that the inhibitory peripheral control of lateral line sense cells by myelinated efferent fibres does not develop in X. laevis until metamorphosis. It is known that during adult leg movements, the level of inhibitory activity in this system rises sharply. One may conclude that adult Xenopus "switches" off its lateral line system during swimming activity. From the present study it is clear that peripheral control of the type seen in the adult is absent in larvae.

It is concluded that the mode of function of the lateralis system is modified during development and that the peripheral changes described necessitate modifications in the central nervous system. It may well be that the principles involved in the development of the lateral line system requiring concurrent change of function apply widely to other sense organ systems.

The work concerns the machanisms of colour vision at the level of the retina in compound eyes. The arrangement of the photoreceptive structures, called rhabdomeres, is not identical in all species. In some (e.g. flies, certain bugs) the several rhabdomeres behind one facet are anatomically distinct, each acting as a separate conduction path for light. In these "open-rhabdom" eyes, the problem is essentially one of correlation of spectral sensitivity measurements taken electrophysiologically by intracellular recording from single photoreceptors with spectral absorption measurements on individual rhabdomeres by microspectrophotometry. This has already been studies elsewhere in the eye of the fly but not in the other animals with open rhabdom eyes.

By far the greatest majority of compound eyes, however, have fused rhabdoms, where, within each omma, structures from several photoreceptors come together along the central axis and give rise to a single fused rhabdom which conducts light axially by internal reflections. This light guide property of the fused rhabdom prevents the effective measurement of the spectral absorption of the individual rhabdomere elements. It is hoped that the problem of whether or not each omma in these cases acts as a spectrally homogeneous unit, will be elucidated by simultaneous electrophysiological recording of the spectral sensitivities of two photoreceptors in the same omma. An attempt is also being made to mark the recording sites by electrophoretic injection of dye through the recording microelectrodes, so that they can be identified histologically. Basically the problem is whether receptors of differing colour sensitivity lie behind one facet or not, because this question must be answered before the further processing of the colour information can be studies at deeper levels.

Tiered retinas: a type of compound eye

(R. Butler)

The ultrastructure and function of tiered retinas in *Periplaneta* and *Pieris* are under investigation. Below each facet of *Pieris* eye are two tiers of four receptor cells each. Electrophysiological techniques are employed to elucidate the role of the upper and the lower cells to reveal the functional significance of the tiered configuration in arthropod compound eyes. The structure is being checked by electron microscopy.

The retina of *Periplaneta*, originally worked out in 1930's by light microscopy is being re-examined by electron microscopy. The retina has a complex tiered form. Intracellular recordings are being made to try to establish two or three distinct populations of cells, and to correlate these to the anatomical findings.

In each case, the responses of each cell to light intensity, polarised light and a variety of monochromatic colours are being used as criterion for evaluating the functional role of that cell in relation to its field of view and position in the eyes.

Neural connection patterns in Arthropod compound eyes

(I.A. Meinertzhagen and G.A. Horridge)

Anatomical pathways and neuronal profiles of the photoreceptors and second-order neurones of the optic lobes of various insects and crustacea were a favourite study of early anatomists. The methods of investigation of these earlier workers, notably Cajal, involves selective staining of entire neurons: a small fraction only are seen at any one time.

Whilst a valuable bachground, this work is inadequate in indicating the exact flow of excitation between the different neuropile layers, knowledge of which is essential to interpret electrophysiological findings. Consequently the routine technique of staining 1 µm sections of plastic embedded tissue has been extended to series of over a thousand sections collected in sequence, and high resolution photomicrographs prepared from each section used to follow the paths of visual axons through the eye. The limit to resolution set by light microscopy is compensated for by the great increase in volume of the tissue which may be analysed compared with analyses of serial electron micrographs.

In this way, the first- and second-order anatomical projections have been analysed for the following insects locust, drone bee, dragonfly and blowfly. Incomplete projections have been analysed in the backswimmer, Notonecta, and analysis of the projections of the bug, Lethocerus, and the crayfish is anticipated.

Two important features emerge from this work. Firstly, fused rhabdomere eyes (locust, bee, dragonfly) in which the photoreceptor cells of one ommatidium receive light from a common optical apparatus, have a first projection in which excitation from these cells is summed at a single locus in the first neuropile or lamina. Open rhabdomere eyes (fly) in which the photoreceptors of one ommatidium have different visual axes, have a first projection which is complex and results in excitation being conveyed to one position in the lamina from all cells (in different ommatidia) that have a common visual axis. (Preliminary observations support a similar system in the aquatic bug Lethocerus). The second-order neurons, in both eye types, result in a comparable point to point projection between the first and second neuropiles, so that all compound eyes so far examined have anatomical projections which accurately reconstruct the visual field of the animal accross the second neuropile. The second feature to emerge is the incredible degree of geometrical order and accuracy in these systems. It confirms in a detailed way the general architecture of all arthropod nervous systems and is in marked contrast to that of vertebrates. This high degree of orderliness makes favourable the analysis of the factors controlling growth and development of the neurons in the visual systems in these animals.

Publications

The group is so recently formed that publications have not yet appeared from the work at A.N.U.

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HORRIDGE, G.A.** and TAMM, S.L.‡ Critical point drying for scanning electron microscopic study of ciliary motion. Science, 163, 817-818 (1969).

HORRIDGE, G.A.** Unit studies on the retina of dragonflies. Zeitschrift fur vergleichende Physiologie, 62, 1-37 (1969).

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SANDEMAN, D.A.** The synaptic link between the sensory and motor axons in the crab eye reflex. Journal of Experimental Biology, 50, 87-98 (1969).

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YOUNG, D.** The motor neurons of the mesothoracic ganglion of Periplaneta americana. Journal of Insect Physiology, 15, 1175-1179 (1969).

WALCOTT, B.**† The ultrastructure and physiology of the abdominal air guide retractor muscles in the giant water bug, Lethocerus. Journal of Insect Physiology, 15, 1855-1873 (1969).

WALCOTT, B.**† Movement of retinula cells in insect eyes on light adaptation. Nature London, 223, 971-972 (1969).