Arnold, John M., William C. Summers, Daniel L. Gilbert, Richard S. Manalis, Nigel W. Daw, and Raymond J. Lasek (1974) *A Guide to Laboratory Use of the Squid Loligo Pealei.* Woods Hole, MA: Marine Biological Laboratory, 74pp.

VII. Squid visual system

Cephalopods have large simple image-forming eyes, which are unlike the compound eyes of most invertebrates. In *Loligo pealei* the eye is about 1 cm across, and takes up a substantial part of the head. There are probably over 10 million receptors in each eye (Young, 1963).

The anatomy of both the eyes and the optic lobes that they project to is intriguing (Young, 1971). The receptors have a rectangular crosssection with microvilli on two opposite faces of their distal segments (Zonana, 1961; Young, 1971; Cohen, 1973). The distal segments are packed together in a tight array and the microvilli in a cell are oriented parallel or perpendicular to those of neighboring cells. This may be related to the detection of the polarization of light. Individual receptors in *Octopus vulgaris,* which are anatomically similar to those of *Loligo pealei,* respond to light polarised in a particular direction, usually either horizontal or vertical on the retina (Tasaki and Tsukahara, 1972). The receptors contain a black pigment, ommin, some of which migrates to the tips of the receptors in bright light and there forms a dense layer. This reduces the amount of light reaching the receptors substantially (Hagins and Liebman, 1962; Young, 1963; Daw and Pearlman, 1974).

The receptors project directly back to the optic lobe. The axons of the receptors are the fibres of the optic nerve, and fire action potentials (MacNichol and Love, 1961; Wurtz, 1961). The optic lobe is a large structure just behind the eye, almost as large as the eye itself. Within the cortex of the optic lobe, layers of cells (nuclear layers) alternate with layers of cell connections (plexiform layers). The core of the optic lobe is occupied by a number of aggregations of cells whose function may not be purely visual (Young, 1971). Golgi impregnations of cells in the layered part of the optic lobe show that they fall into groups, defined by the arrangement of the processes of the cells (Young, 1973). The processes of some cells end in a long narrow area in the outermost plexiform layer, whereas other cells have only a few processes ending in a small area near the surface of the lobe. The optic lobe also contains cells which send their processes back to the retina. These are therefore efferent fibers (Young, 1971; Cohen, 1973).

Golgi methods (Young, 1971, 1973) and electron microscopy (Cohen, 1973) have been used to analyse these connections. The density of the optic lobes of cephalopods is such that fixation by perfusion rather than by immersion is essential for electron microscopy. These perfusions are readily carried out by cannulation of the systemic heart. Whereas the retina is perfused in this procedure the tall distal segments of the visual cells occur in a non-vascular region. It is therefore useful to inject the eye with additional fixative after the perfusion has started. (Cohen, 1973).

In some respects the squid has proved to be a very useful animal in which to study the physiology and biochemistry of vision. In general, preparations devised to study receptor processes and the initial absorption of light by the visual pigment have been successful, while preparations devised to study the physiology of the visual system at higher levels have not been so fruitful.

The success of preparations for studying receptor processes is due to the fact that most of the cells in the retina are receptor cells, that the distal parts of the photoreceptors, which contain the visual pigment, can be split off from the rest of the retina with very little contamination, and that slices of retina will survive for hours

in oxygenated sea water. For studying the visual pigment of the squid (rhodopsin), the retina is frozen. This helps to detach the distal segments of the receptor, containing the visual pigment, from the cell bodies of the receptor cells and the supporting cells. A series of fractionations removes contaminating material from the distal segments, then the visual pigment is extracted with digitonin. The whole procedure must be carried out close to 0°C and under red light, to avoid any degradation of the visual pigment. Details of the procedures are given in Hubbard and St. George (1958). Characteristics of the protein component of the pigment have been worked out by F. J. M. Hagins (1973).

Pioneering work on squid rhodopsin showed that absorption of light converts it to metarhodopsin but does not bleach it (Krukenberg, 1882; Bliss, 1942-3; Wald, Durrell and St. George, 1950; Hubbard and St. George, 1958). In other words the reaction stops at metarhodopsin and does not proceed to retinal + opsin, as it does in the vertebrate. The metarhodopsin exists in two forms, acid metarhodopsin and alkaline metarhodopsin, according to the pH (Hubbard and St. George, 1958). Both forms are converted back to rhodopsin by the absorption of light, but with different spectral sensitivities. This pattern of conversion of rhodopsin to metarhodopsin involves the conversion of the chromophore, retinal, from the 11-cis to the all-trans form. The squid retina contains a second pigment, retinochrome, located in the somal region of the receptors rather than the distal segments. This pigment contains all-trans retinal which is converted by light to 11-cis retinal (Hara and Hara, 1972). Retinochrome may therefore play a part in the regeneration of squid rhodopsin. Retinochrome is extracted from the remainder of the retina after the distal segments have been removed, by taking off the front half of the eye and stirring the back half violently upside down in a solution of neutral phosphate buffer. Details of subsequent procedures are given by Hara and Hara (1972).

The local origin of the receptor current was first established in the squid retina (Hagins, et al., 1962; Hagins, 1965). For this experiment, the retina is cut into slices of 100-300u parallel to the length of the receptors and viewed under infrared light, while electrodes are pushed into it. The slice is mounted on the cooled stage of an inverted microscope, with an image converter attached, and the infrared light outlines the receptor distal segments and electrodes, while the area illuminated by visible light can also be seen. With an array of three or more electrodes, it is possible to measure the current flow resulting from illumination of a small area of the distal segments of the receptors, and to see how the current flow varies when the spot of light is moved. Two factors which make this experiment feasible are the length of the distal segments of the receptors (about 300u) and the uniformity of the receptor population. The current flow acts as though current moves out across the receptor membrane from all parts of the distal segments and back in to the receptor in the illuminated area. In other words, illumination of a limited part of a receptor leads to changes in the membrane in the same limited area. This appears to be true for vertebrate photoreceptors as well as squid, and is probably true for all photoreceptors in which the visual pigment is associated with the cell membrane.

Slices of retina can also be used to investigate a fast photovoltage which is correlated with conversion of rhodopsin to metarhodopsin and back again (Hagins and McGaughy, 1967, 1968) and can therefore be used as an indicator of the state of the visual pigment. This fast photovoltage is recorded across the retina in retinas fixed with glutaraldehyde, which eliminates the large slow photocurrents. Precautions must be taken to shield the electrode from light, to reduce electrical artifacts

from the flash of light, and to correct for the thermoelectric voltage produced by the light.

Two preparations have been used in attempts to analyze the function of the optic lobes in the squid. In one preparation the intact squid is held down on a board by four pins (Wurtz, 1961; Daw and Pearlman, 1969). Some of the skin near the optic lobes is dissected away, then an electrode is pushed through the remainder of the skin without, if possible, opening the space between the eye and the lobe, which is a venous sinus. The main problem with this preparation is that the squid moves periodically, dislodging the electrode, and no drug has been found which will stop the squid from moving without killing it. For this reason a second preparation has been devised, in which the eye and optic lobe are dissected and put into a perfusion solution (Hartline and Lange, 1973). Details of this preparation have not yet been published and at present the function of the fascinating anatomical structure of the squid optic lobe is still a mystery.

REFERENCES:

BLISS, A. F., 1942-43. Derived photosensitive pigments from invertebrate eyes *J. Gen. Physiol.* 26:361-367.

COHEN, A. I., 1973. An ultrastructural analysis of the photoreceptors of the squid and their synaptic connections. *J. Comp. Neurol.* 147:351-426.

DAW, N. W. AND PEARLMAN, A. L., 1969. Receptive field studies of single units in the optic lobe of the squid, *Loligo pealei. Biol. Bull.* 137:398.

DAW, N. W. AND A. L. PEARLMAN, 1974. Pigment migration and adaption in the eye of the living squid, *Loligo Pealei. J. Gen. Physiol.* In press.

HAGINS, F. J. M., 1973. Purification and partial characterisation of the protein component of squid rhodopsin. *J. Biol. Chem.* 248:3298-3304.

HAGINS, W. A., 1965. Electrical signs of information flow in photoreceptors. *Cold Spring Harb. Symp. Quant. Biol.* 30:403-418.

HAGINS, W. A. AND P. A. LIEBMAN, 1962. Light induced pigment migration in the squid retina. *Biol. Bull.* 123:498.

HAGINS, W. A. AND MCGAUGHY, R. E., 1967. Molecular and thermal origins of fast photoelectric effects in the squid retina. *Science* 157:813-816.

HAGINS, W. A. AND MCGAUGHY, R. E., 1968. Membrane origin of the fast photovoltage of squid retina. *Science* 159:213-215.

HAGINS, W. A., H. V. ZONANA AND R. G. ADAMS, 1962. Local membrane current in the outer segments of squid photoreceptors. *Nature* 194:844-847.

HARA, T. AND R. HARA, 1972. Cephalopod retinochrome. In *Handbook of Sensory Physiology* Vol. VII. 1 ed. H. J. A. Dartnall pp 720-746 (Springer, Berlin).

HARTLINE, P. AND D. LANGE, 1973. Private Communication.

HUBBARD, R. AND R. C. C. ST. GEORGE, 1958. The rhodopsin system of the squid. *J. Gen. Physiol.* 41:501-528

KRUKENBERG, C. F. W., 1882. Ueber die Stabchenfarbe der Cephalopoden *Untersuch. Physiol. Imt. Univ. Heidelberg.* 2:58.

MACNICHOL, E. F. AND W. E. LOVE, 1961. Impulse discharges from the retinal nerve and optic ganglion of the squid. In *Neurophysiology and Psychophysics of the Visual System;* ed. R. Jung and H. Kornhuber, p. 97-103 (Springer, Berlin).

TASAKI, K. AND Y. TSUKAHARA, 1972. Octopus retina as an analyzer for polarized light. *Proc. Austral. Physiol. Pharm. Soc.* 3:2.

WALD, G. J. DURRELL AND R. C. C. ST. GEORGE, 1950. The light reaction in the bleaching of rhodopsin. *Science* 111:179.

WURTZ, R., 1961. Optic nerve responses of squid, *Loligo pealei. Biol. Bull.* 121:414-5.

YOUNG, J. Z., 1963. Light and dark adaptation in the eyes of some cephalopods. *Proc. Zool. Soc.* 140:255-723.

YOUNG, J. Z., 1971. *The anatomy of the nervous system of Octopus vulgaris* (Oxford Univ. Press).