Intracellular Absorption Difference Spectrum of Limulus Extra-Ocular Photolabile Pigment

Abstract. *Microspectrophotometric measurements on single cell bodies located on the surface of the "lateral olfactory nerve" of* Limulus polyphemus *show they contain a photolabile pigment with an absorption-difference spectrum similar in shape to that of most other visual pigments and with a maximum absorption at 529 ± 5 nanometers.*

Recent investigations indicate the existence of apparent photoreceptors located in the "lateral olfactory nerves" of *Limulus polyphemus* (*1*). These nerves are located on either side of the ventral midline, running forward from the circumesophageal ganglion to a small "olfactory wart" on the ventral surface, about a centimeter forward of the mouth. Large cell bodies scattered along this nerve, referred to by earlier workers as the ventral eye (*2*), behave in most respects like the retinular cells of the lateral compound eye (*1*). Unlike the retinular cells, however, they contain no dense screening pigments, and, thus, intracellular measurement of the photolabile pigments contained in them can be made. Absorption-difference spectra were recorded from several lateral olfactory nerve cells with a dual-beam microspectrophotometer developed by the author.

This new microspectrophotometer is a modified version of the one originally developed and used by Marks (*3*). A small beam of monochromatic light is chopped by a rotating mirror to pass alternately along two identical optical paths, one of which contains the specimen; the other contains a blank slide. Each path contains two microscopes, an inverted one which images the light pattern (1 μ to 400 μ across) on the specimen plane (or blank slide) and an erect one which collects the transmitted light. By a second encounter with the rotating mirror the transmitted fluxes from the two paths are passed alternately onto a sensitive photomultiplier tube (EMI 9558A). Successive signals from the photomultiplier are amplified and synchronously switched into two separate channels. A digital voltmeter automatically takes the ratio of these two signals, records it on IBM punch-paper tape for computer analysis, and plots this ratio as a function of the wavelength on an *x-y* plotter for monitoring during the experiment (Fig. 1). The light source is servo-controlled to hold constant the quantum flux output of the monochromator. Two additional light sources in the microspectrophotometer can image patterns on the specimen, either during or between measurements of transmission, to control the bleaching or to allow observation of the specimen.

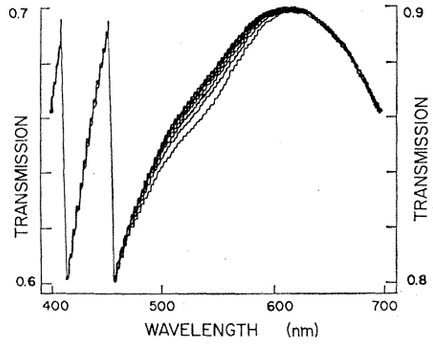

Fig. 1. Monitor plot of transmissions recorded during one experiment on a single cell in an olfactory nerve of *Limulus*. The transmission is plotted vertically, with a full-scale excursion representing a change in transmission of 0.1 and with the origin reset automatically every 0.1 transmission unit. The horizontal motion is slaved to the microspectrophotometer monochromator which scans linearly in wavelength. As bleaching occurs the curve is seen to rise. In this experiment, 36 percent of the available pigment was bleached between scans, and the total change in optical density, one of the largest, was 0.015.

The ratios of transmitted flux are determined at frequent intervals throughout the visible range of wavelengths; this represents the transmission spectrum of the specimen. Such transmission spectra are measured before and after a bleaching of the pigment; their difference is the absorption-difference spectrum of the photolabile pigments contained in the specimen.

Lateral olfactory nerves from mature *Limulus* were dissected and desheathed in deep-red light and sealed in sea water–agar gel between two cover slips. The slides were placed in the microspectrophotometer and viewed under infrared light while a cell body was positioned over a measuring beam 20 μ in diameter. Transmissivities at 101 wavelengths, evenly spaced between 400 nm and 700 nm, were recorded initially and after each of a series of bleaches until no further bleaching occurred. From the digitalized records, I computed the absorption-difference spectra on the IBM 7094, making corrections for the unavoidable bleaching caused by the measuring light during each scan (*3*, *4*). Typically, bleaches between scans reduced the pigment present by about one-third. Changes in total optical density were between 0.005 and 0.015 log units. In these experiments the noise corresponds to approximately 5×10^{-4} change in transmission.

Data for 16 cells were averaged and plotted by the computer (see Fig. 2). Filled circles give the mean values of the absorption difference, and the dashes represent the standard errors in those means. For comparison, the superimposed solid line shows a Dartnall-template curve (*5*). This empirical curve fits closely the shape of most extinction spectra of visual pigments when plotted as a function of the wave number. The best fit by eye to the above data for the olfactory nerve cells occurs when this curve is positioned with its maximum at 529 ± 5 nm. The good agreement over most of the range suggests that the pigment measured is similar to most other rhodopsin-like visual pigments. Although the increased absorption near 420 nm is probably not significant, the fact that the difference spectrum is positive at short wavelengths suggests that photoproducts are not prominent.

The open circles show the electrophysiological action spectra reported for these olfactory nerve cells (*1*), with standard errors indicated. The maximum values for the action spectra and the absorption-difference spectra of these cells are close to that of the absorption spectrum reported for visual pigment extracted from the lateral eye,

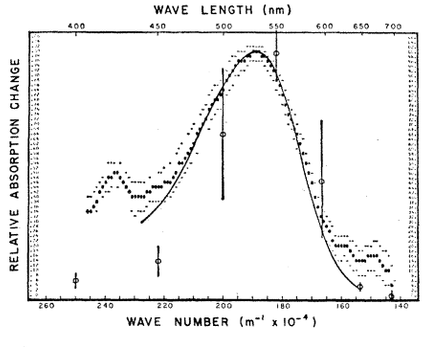

Fig. 2. Absorption-difference spectra of photoreceptor cells from *Limulus* olfactory nerve. See text for explanation of symbols.

which has a maximum at 520 nm (*6*). They agree well with the secondary peak at 530 nm in the spectral sensitivity of the median ocellus of *Limulus*. This peak is suggested to result from a pigment distinct from the one responsible for the main peak at 360 nm (*7*). Recordings of single cells show that the lateral eye contains two cell types —alpha cells with maximum sensitivity at 525 nm and beta cells with broad sensitivity from 550 nm to 350 nm (*8*). The photopigment of the alpha cells may be the same as the one reported here for the photoreceptor cells of the lateral olfactory nerve.

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Periventricular Cerebral Impedance after Intraventricular Injection of Calcium

Abstract. *Injection of small volumes of calcium solution into the lateral ventricle of the cat was followed by large electrical-impedance changes in gray matter bounding the ventricle, including the caudate nucleus and hippocampus. These changes lasted more than 24 hours and were accompanied by epileptiform electroencephalographic activity. Biweekly injections led to status epilepticus. Injections of similar amounts of magnesium ions were without comparable effects. Possible interactions between calcium ions and intercellular macromolecular material are discussed as a basis for certain impedance shifts in cerebral tissue.*

Impedance-measuring currents applied to cerebral tissue appear to pass mainly through intercellular fluid (*1*) and perhaps also through neuroglial cells. The role of these cells as a substantial pathway for current remains uncertain, but low resistance of the neuroglial membrane has been reported in tissue culture (*2*). Neuronal membrane resistance has been repeatedly estimated at 4 to 7000 ohm cm^2 in the mammal, at least several orders of magnitude greater than resistance of intercellular fluid (*3*).

Impedance changes in cerebral tissue have been detected in the course of alerting, orienting, and discriminative responses (*4*). Nevertheless, the origin of impedance changes accompanying physiological responses has remained obscure. Electron micrographs prepared with glycol solutions (*5*) showed a substantial content of macromolecules, possibly mucopolysaccharides, in cerebral extracellular fluid. This suggested to us that divalent cations, such as calcium, may be important in regulation of macromolecular configurations at the neuronal surface, thus influencing ionic fluxes that determine neuronal excitability and also modulate conductance characteristics in perineuronal fluid.

Impedance measurements were made in six cats with coaxial electrodes chronically implanted bilaterally in the caudate nucleus, dorsal hippocampus, and amygdala. This technique employed a small measuring signal with an amplitude of 20 μv and a typical current density of 10^{-13} amp per square micron of electrode surface at 1.0 khertz and with the tissue in one leg of a Wheatstone bridge. More than 90 percent of the current passed within a volume of 1.0 mm^3 surrounding the electrode tip. With this method, the residual bridge signal was amplified and sampled through two gated pulse "windows" placed in in-phase and quadrature (90° displaced) positions relative to the 1.0-khertz sine-wave test signal (*4*). The integrated output of signals admitted through these windows provided a measure of relative resistive and reactive components of tissue impedance. This coherent detection method is extremely sensitive to small phase-locked components of amplified output of the bridge. Our previous studies indicated that impedance responses accompanying physiological stimuli do not arise in simple relation to such factors as cerebral blood pressure, cerebral blood flow, or tissue temperature, but appear to relate directly to physiological phenomena requiring the presence of normal neural elements (*4*, *6*).

After a postoperative interval of 30 days for impedance base line stabilization, injections of calcium chloride solution (40 or 60 μeq in 0.1 ml) were made through an intraventricular cannula, preceded by injections of an equal volume of normal saline (Fig. 1). No changes followed the saline injections. A sharp decline occurred in both resistive and reactive components, beginning 15 to 30 minutes after injection of 40 to 180 μeq of calcium solution, in the structures which bound the lateral ventricle.

In some cases impedance readings were shifted from base line values by as much as 25 percent for periods that exceeded 36 hours. Maximum shifts occurred within 2 hours of injection, with slow return to base line thereafter. Changes were largest and earliest in structures closest to the tip of the cannula and were delayed 30 to 50 minutes in reaching peak values in symmetrically placed leads in the opposite half of the brain. Injection of calcium into the ventricle was regularly followed by a topographically determined sequence of impedance changes, consistent with diffusion from the injection site through the cerebrospinal fluid, but direct injection of calcium solutions in doses up to 120 μeq into periventricular structures was without comparable effects, except at electrodes immediately adjacent to the injection site.

Onset of impedance shifts in hippocampus and amygdala was accompanied by seizure-like discharges. However, impedance shifts were prolonged for many hours beyond the cessation of gross electroencephalographic abnormalities. In animals given 40 to 100 μeq of calcium at intervals of 2 weeks, status epilepticus resulted after three or four injections, with generalized convulsions leading to death.

Although these findings strongly suggest effects attributable to calcium, other factors require consideration. Changes due to altered volume of cerebrospinal