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City University of New York PH.D. 1982

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ELECTROPHYSIOLOGY AND PHARMACOLOGY OF A LOCAL CIRCUIT FEEDBACK SYSTEM IN NEURONS OF THE OCELLAR RETINA

by

Susan L. Stone

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1981

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1982

Susan Stone

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

ELECTROPHYSIOLOGY AND PHARMACOLOGY OF A LOCAL CIRCUIT FEEDBACK SYSTEM IN NEURONS OF THE OCELLAR RETINA

by

Susan L. Stone

Advisor: Professor Richard L. Chappell

Intracellular electrophysiological and pharmacological studies of photoreceptor and L-neuron (second order neuron) responses in the dragonfly ocellar retina suggest the hyperpolarizing OFF transient in the receptor reflects synaptic feedback from L-neuron dendrites onto receptor terminals. The receptor OFF response was normally observed when recording more proximally, closer to the nuclear and synaptic regions but was not seen when recording more distally, closer to the rhabdomeric end of the cell. Both the hyperpolarizing OFF response in the receptor and the depolarizing OFF response in the L-neuron are apparently generated in the ocellar plexiform layer because they were not eliminated when the L-neuron processes were isolated from the brain by severing the ocellar nerve. Direct evidence for synaptic feedback onto receptor terminals is provided by antidromic stimulation experiments, in which a normal-appearing OFF transient was recorded intracellularly from the dark-adapted receptor in response to the application of brief hyperpolarizing current pulses to the ocellar nerve, and pharmacological studies in which the responses of ocellar retinal neurons (in intact and cut nerve preparations) were modified by various drugs known to interfere with synaptic transmission. Cholinergic agonists mimicked some effects of light on the receptor and L-neuron response, curare reduced or blocked the receptor OFF

response and eliminated all activity in the L-neuron, and picrotoxin increased the magnitude of the OFF response in both types of ocellar neurons. These findings are consistent with a sign-conserving feedback model and support the view that the receptor transmitter may be acetylcholine and the feedback (L-neuron) transmitter could be GABA. In addition, some of the results suggest that lateral synaptic interactions between photoreceptors and L-neuron dendrites may also contribute to the observed changes in the waveform of ocellar retinal responses.

Spontaneous fluctuations in dark potential were commonly observed in intact nerve preparations following exposure to drugs, and in cut nerve preparations untreated with drugs. In photoreceptors, the dark potential appeared to fluctuate between two distinct dark equilibrium levels. Such spontaneous dark oscillatory behavior may represent disruption of the dark equilibrium of a local circuit feedback loop whose stability is perturbed by pharmacological manipulation or by isolating L-neuron processes from the brain.

To Paul, Lisa and Louis; for putting up with me.

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INTRODUCTION

Synaptic interactions within the retina are currently of special neurophysiological interest because they involve graded synaptic transmission (synaptic transmission mediated by slow potentials, not action potentials) between neuronal processes organized in local circuits (Werblin and Dowling, 1969; Chappell, 1970; Chappell and Dowling, 1972; Schmitt et al., 1976; Dowling, 1979; Shaw, 1979). The median ocellus of the dragonfly is particularly well suited to the study of synaptic transmission in the retina since, as far as is known, it contains just two types of neurons (photoreceptors and second order neurons) which synapse in a single plexiform layer that is well isolated from the brain (Cajal, 1918; Ruck and Edwards, 1964; Dowling and Chappell, 1972). Therefore, slow potential mediated synaptic transmission between photoreceptors and second order cells can be examined without the added complexities due to the activity of higher order neurons.

An ocellus is a simple cup-shaped eye that is found in many insects in addition to their large, multifaceted compound eyes (Cajal, 1918; L.J. Goodman, 1975; Wilson, 1978a). Optical measurements made on those ocelli examined to date indicate that the ocellus is underfocussed and does not act as an image forming device (L.J. Goodman, 1975; Wilson, 1978a). The high degree of convergence of receptor cells onto a few large second order neurons ("L-neurons" after Martin Wilson, 1978a) suggests the ocellus functions as a sensitive light detector (Cajal, 1918; Chappell and Dowling, 1972; Dowling and Chappell, 1972; L.J. Goodman et al., 1979).

The median ocellus of the dragonfly consists of a layer of photoreceptor cells, a synaptic zone in which the axons of the photoreceptors come into synaptic contact with the dendrites of L-neurons, and the ocellar nerve which leads from the eye to the brain (Cajal, 1918; Ruck and Edwards, 1964; Chappell and Dowling, 1972; Dowling and Chappell, 1972). The ocellar nerve is formed by the processes of seven large second order neurons (L-neurons) and about 40 smaller second order processes (Chappell et al., 1978). The electrophysiological properties of the smaller ocellar nerve fibers have not been established.[1] L-neurons branch extensively within the ocellar neuropil and also arborize extensively in the brain, where their cell bodies are located (Kirkham et al., 1975; Chappell et al., 1978; Patterson and Chappell, 1980; Mobbs et al., 1981). Photoreceptors are 15 microns in diameter and 300-400 microns in length (Chappell and Dowling, 1972; Patterson and Chappell, 1980), and the largest L-neuron dendrites are 15-20 microns in diameter (Dowling and Chappell, 1972; Kirkham et al., 1975; Patterson and Chappell, 1980; Mobbs et al., 1981). Approximately 1500 retinula cells synapse with L-neuron dendrites in the plexiform layer (Cajal, 1918).

The first intracellular responses from photoreceptors and L-neurons in an insect ocellus were recorded in the dragonfly by Chappell (1970) and Chappell and Dowling (1972), who found that the photoreceptor responds to light with a graded depolarization, while the L-neuron responds to light with a graded (more phasic) hyperpolarization. Spectral sensitivity studies of receptor (Chappell and Devoe, 1975) and L-neuron (Mobbs et al., 1981) responses reveal maximal sensitivity in the ultraviolet and green regions of the spectrum.

The general waveform of light responses of ocellar retinal neurons in the dragonfly resemble photoreceptor and second order responses in the median ocelli of the locust (Patterson and Goodman, 1974) and barnacle (Stuart and Oertel, 1978; Oertel and Stuart, 1981) and the compound eyes of insects (locust: Shaw, 1968; fly: Scholes, 1969; Jarvilehto and Zettler, 1971; dragonfly: Laughlin, 1973).

Synaptic transmission between photoreceptors and L-neurons in the dragonfly median ocellus is mediated by slow potentials (Dowling and Chappell, 1972), which is the primary mode of synaptic transmission in the vertebrate retina (Bortoff, 1964; Tomita, 1965; Werblin and Dowling, 1969) and many invertebrate retinas (Burkhardt, 1962; Gwilliam, 1963, 1965; Shaw, 1968, 1972; Jarvilehto and Zettler, 1970, 1971; Scholes, 1969; Laughlin, 1973; Patterson and Goodman, 1974; Hudspeth and Stuart, 1977; Stuart and Oertel, 1978; Wilson, 1978b; Guy et al., 1979).

In a number of invertebrate visual systems, it has been suggested that during illumination the depolarized photoreceptors release an inhibitory neurotransmitter that hyperpolarizes second order neurons by increasing conductance to an ion or ions with a negative equilibrium potential (Shaw, 1968; Chappell, 1970; Chappell and Dowling, 1972; Laughlin, 1974b; Klingman, 1976; Klingman and Chappell, 1978; Wilson, 1978b; Zimmerman, 1978; Stuart and Oertel, 1978; Oertel and Stuart, 1981). On the other hand, vertebrate photoreceptors (rods and cones) hyperpolarize during illumination (Bortoff, 1964; Tomita, 1965; Toyoda et al., 1969; Werblin and Dowling, 1969; Baylor and Fuortes, 1970) and stop releasing an excitatory neurotransmitter on to second order cells

(horizontal cells and hyperpolarizing bipolar cells) causing such second order cells to hyperpolarize because conductance to an ion or ions with more positive equilibrium potential has decreased (Toyoda et al., 1969; Nelson, 1973; Trifonov et al., 1974). Consequently, although second order neurons in both invertebrates and vertebrates hyperpolarize during illumination, they do so because the sign of the photoreceptor light response and the action of the receptor transmitter is opposite in each case.

Although the dragonfly ocellar retina is relatively simple compared to the insect compound eye (Strausfeld and Campos-Ortega, 1977; Shaw, 1979) and the vertebrate eye (Dowling and Boycott, 1966; Dowling and Werblin, 1969), electronmicroscopic studies reveal a surprisingly complex organization of lateral, reciprocal, and feedback synapses, in which receptor terminals and L-neuron dendrites were described as both presynaptic and postsynaptic elements (Dowling and Chappell, 1972). The majority of the synaptic contacts observed were of the dyad type, with one presynaptic process associated with two postsynaptic elements. When the presynaptic element in the dyad was a receptor terminal, the two postsynaptic processes could be either receptor terminals, L-neuron dendrites, or one of each, and the same was true when the L-neuron dendrite was the presynaptic element of the dyad. In some cases, an L-neuron dendrite was seen to be pre- and postsynaptic to the same receptor terminal. The dyad synapses in the dragonfly ocellar retina are similar to the dyad synapses in the inner plexiform layer of the ve e-brate retina (Dowling and Boycott, 1966; Dowling and Werblin, 1969 nd the retina of several invertebrate species (Truijillo-Cenoz, 1965;

Hamori and Horridge, 1966; Whitehead and Purple, 1970; Armett-Kibel et al., 1977; L.J. Goodman et al., 1979). In the fly compound eye the possibility of feedback synapses from second order neurons (L2) onto receptors has not been ruled out (Strausfeld and Campos-Ortega, 1977), but in the case of ocelli, anatomical evidence for feedback synapses from second order neurons onto receptor terminals has only been described in the dragonfly (Dowling and Chappell, 1972) and the locust (L.J. Goodman et al., 1979).

A particularly interesting feature of the intracellular responses of both types of dragonfly ocellar neurons is a prominent oscillatory wave following light-OFF (Chappell and Dowling, 1972). Upon termination of the light stimulus, the L-neuron response shows a depolarizing OFF-transient, while the photoreceptor response shows a hyperpolarizing OFF-transient. Such OFF responses may be of behavioral significance to animals with ocelli because extracellular impulse activity can be recorded from the dragonfly ocellar nerve (Ruck, 1961a,b,c; Rosser, 1974; Kondo, 1978) and the ventral nerve cord of the locust (Patterson and Goodman, 1974b) upon termination of the light stimulus. In the barnacle, the dimming of light mediates the "shadow reflex" causing the animal to withdraw into its shell (Gwilliam, 1963, 1965; Millecchia and Gwilliam, 1972; Ozawa et al., 1975).

Although a depolarizing OFF response in the second order neuron has been observed in several invertebrate visual systems (Shaw, 1968; Scholes, 1969; Autrum et al., 1970; Jarvilehto and Zettler, 1971; Laughlin, 1973; Menzel, 1974; Patterson and Goodman, 1974; Ozawa et al., 1975; Erber and Sandeman, 1976; Stuart and Oertel, 1978; Guy et al., 1979; Oertel and Stuart, 1981), the hyperpolarizing OFF response character-

istic of the dragonfly ocellar receptor response has not been reported in other species. It has been suggested that the transient OFF responses recorded from dragonfly ocellar retinal neurons might be generated by synaptic interactions in the ocellar plexiform layer; in particular, the hyperpolarizing OFF response in the photoreceptor might be the result of synaptic feedback from L-neuron dendrites onto receptor terminals (Chappell and Dowling, 1972; Dowling and Chappell, 1972).

Subsequent electrophysiological and pharmacological studies of dragonfly L-neuron responses support this idea. Klingman and Chappell (Chappell and Klingman, 1974; Klingman, 1976; Klingman and Chappell, 1978) found that the L-neuron response could be selectively modified by various drugs known to interfere with synaptic transmission and developed a model to account for their pharmacological findings. Briefly, they proposed that L-neuron dendrites release a feedback transmitter onto receptor terminals when the L-neurons are depolarized in the dark. They suggested that the action of the feedback transmitter was to facilitate the release of an inhibitory transmitter from receptor terminals and that the receptor transmitter may be acetylcholine and the feedback transmitter could be gamma-aminobutyric acid (GABA). The finding that significant amounts of acetylcholine and choline acetyltransferase are present in the dragonfly median ocellus (Chappell and Kuhar, 1976; Chappell, 1979) is consistent with the above hypothesis. Martin Wilson (1978c) has also suggested that L-neuron dendrites might release a facilitatory neurotransmitter onto receptor terminals in the locust median ocellus, although his interpretation was based on electrophysiological studies of L-neuron responses and has not been confirmed by direct recording from photoreceptors.

Recent evidence suggests that synaptic feedback onto photoreceptors may be an important local circuit mechanism responsible for the processing of visual information in the vertebrate retina (Baylor et al., 1971; Lam et al., 1979; Murakami et al., 1978; Piccolino and Gerschenfeld, 1977, 1980; Gerschenfeld and Piccolino, 1980). However, studies desçribing pharmacological modification of vertebrate photoreceptor responses are limited and generally inconclusive, and there are no reports in invertebrates in which a feedback model was tested directly by intracellular recording from photoreceptors.

The objective of my thesis experiments was to investigate the feedback model developed for the dragonfly ocellus (Chappell and Klingman, 1974; Klingman, 1976; Klingman and Chappell, 1978) by employing single electrode electrophysiological techniques to record intracellular responses from ocellar retinal neurons. I attempted to answer the following questions:

(1) Will the response of the photoreceptor be modified by the same drugs that were shown to modify the response of the L-neuron?
(2) Is the depolarizing OFF-transient in the L-neuron response and the hyperpolarizing OFF-transient in the photoreceptor response generated in the ocellar plexiform layer or at some other site in the brain?
(3) Are the OFF-transients observed in intracellular responses of ocellar retinal neurons a consequence of synaptic transmission?

During the course of my experiments I found that a portion of my data could not be interpreted solely in terms of a model involving feedback interactions alone. Consequently, I have suggested a revised model

which considers lateral synaptic interactions between photoreceptors and L-neurons as well as feedback interactions from L-neuron dendrites onto receptor terminals. This model is shown in figure 83 and is discussed at length in the text. Although my thesis was not intended to confirm the revised model, it did prove valuable in interpreting the pharmacological data and raised a number of questions which can be tested experimentally in the future. The experimental evidence supporting such a model is presented in the following sections.

METHODS

Preparation

Experiments were performed during the spring of 1977, 1978, and 1979 on 98 laboratory reared dragonflies raised according to the procedure developed by Chappell (1970) from nymphs supplied by Connecticut Valley Biological Supply Company, Southampton, Massachusetts. Nymphs were kept in aquarium tanks divided into individual compartments by perforated plastic dividers and wire screens in order to prevent cannibalism and were fed daily on tubifex worms. The animals were provided with 12 hours of light daily and water temperature was controlled at 20-23° C. Adult dragonflies were used between 24-72 hours after emerging.

All records presented here were recorded from *Aeschna tuberculifera*, but similar intracellular responses were observed in 5 experiments using *Anax junius*. Although I never recorded from wild-caught animals, Chappell (1970), Klingman (1976), and Mobbs et al., (1981) have noted no differences between the responses of wild animals (total of 9 species) and those raised in the laboratory. In addition, I noted no obvious differences in the responses of male or female animals. In my experiments approximately 70% of the dragonflies were male and 30% were female.

Dissection

Each adult dragonfly was decapitated and the head was waxed, frons uppermost, to a small wooden platform. The frons and underlying air sacs were removed to expose the receptor structure and ocellar nerve *in situ* according to the method of Chappell (1970) and Chappell and Dowling (1972). The simple dissection procedure did not damage the ocellus or brain (it was not necessary to touch the ocellus or brain in order to

expose these structures) and allowed the head capsule itself to be used as a perfusion chamber. The optics of the system were preserved because the ocellar retina remained attached to the lens. In 18 experiments the ocellar nerve was severed prior to recording by cutting close to the brain with small iris scissors (Edward Weck and Co. Inc., Long Island City, New York). Only one snip of the scissors was required. After the ocellar nerve was severed, the ocellus and distal stump of ocellar nerve were observed to separate from the brain and float downward in the pool of Ringer until the ocellus came to rest upon the small air sacs underneath. Severing the ocellar nerve did not prolong the dissection time which averaged about 10 minutes.

All experiments were performed on animals which were in a relatively light-adapted condition. The animals were maintained in room light prior to decapitation and the dissection was performed under white light with the aid of a dissecting lamp.

Perfusion Apparatus

The dissected head was mounted with Tackiwax on a small, obliquely cut cork (ocellus pointing down) in the center of a small plexiglass chamber as described by Klingman (1976) and Klingman and Chappell (1978).

In the earlier experiments (approximately 30%) each drug was started by positioning the perfusion needle at the time drug application was initiated and removing the perfusion needle when drug application was stopped. This procedure was subsequently abandoned since it increased the risk of losing the cell because of the mechanical disturbance involved in opening the door of the Faraday cage and moving the perfusion needle close to the ocellus. In addition, in the earlier perfusion experiments the flow rate of the various solutions could not be observed

without opening the door of the cage because the solutions were held in 10 ml syringes mounted on clamps inside the cage (Klingman, 1976).

In the modified procedure each solution was contained in a separate 150 ml plastic chamber (Travenol, Buratrol microdrip; 60 drops per ml) mounted on top of the Faraday cage so that the drip rate could be observed (with a flashlight) during an experiment without opening the door of the cage. Plastic tubings from the chamber were run to the inside of the cage through a small hole in the back and connected to a plastic 3-way stopcock which was mounted on a manipulator positioned close to the plexiglass chamber. A 30 gauge three inch long needle (Becton-Dickinson) was connected to the common outlet of the stopcock and served as the perfusion needle from which droplets of solution flowed into the head capsule, and several such devices could be arranged around the plexiglass chamber. Before the start of an experiment, the perfusion needle was positioned with the manipulator so that the tip rested on the hard cuticle inside the head capsule on either side of the ocellus. The tip of the needle was usually underneath the meniscus of fluid which filled the head capsule to avoid mechanical disturbance from the droplets of solution falling on top of the ocellus and microelectrode. There were several advantages to this type of perfusion apparatus: 1) By leaving the stopcock close to the preparation open, various drug solution changes could be accomplished without opening the cage door because each solution could also be started or stopped by adjusting a ball valve attached to the tubing on the drip chamber above the cage. 2) There was virtually no dead space between the point where the perfusion tubing entered the stopcock and the tip of the 30 gauge needle in the head capsule. 3) There was no chance of cross contamin-

ation of the solutions. 4) The drip rate could be continuously monitored without disturbing the preparation. All experiments were carried out under continuous perfusion with either Ringer solution or a drug dissolved in Ringer at a rate of 6-12 drops per minute (60 drops per ml). Small slivers of twisted Kimwipes (Kimberly-Clark) served as wicks to carry the overflow from the head capsule to the plexiglass chamber and, if necessary, from the chamber to a petri dish which held the chamber. The silver chloride reference electrode made contact with the pool of Ringer in the chamber through a small hole in the side.

Solutions

The Ringer solution used had the following composition: 134 mM NaCl, 5.4 mM KCl, 3.8 mM $CaCl_2$, 3.7 mM $MgCl_2$, 0.5 mM $NaHCO_3$, pH 7.8 at 21^0 C. The ionic composition of the Ringer was based on an analysis of *Aeschna* nymph hemolymph by Duchateau, Florkin, and Leclerq (1953).

Drugs were dissolved directly in the Ringer solution. Drugs used and their sources were as follows: acetylcholine chloride, Pfaltz and Bauer Inc., Flushing, New York; curare (d-tubocurarine chloride), Mann Research Laboratories, New York, New York and Sigma Chemical Company, St. Louis, Missouri; edrophonium chloride, Hoffman-LaRoche, Inc., Nutley, New Jersey; eserine sulfate (physostigmine sulfate), Sigma Chemical Company, St. Louis, Missouri; carbachol (carbamylcholine chloride), Sigma Chemical Company, St. Louis, Missouri; picrotoxin, Sigma Chemical Company, St. Louis, Missouri; cobalt chloride, ("Baker Analyzed" reagent), J.T. Baker Chemical Company, Phillipsburg, New Jersey; ouabain (strophanthin-G), Sigma Chemical Company, St. Louis, Missouri.

All solutions were allowed to equilibrate to room temperature (21° C) before use.

Intracellular Recording

Glass microelectrodes were drawn on a Narishige PD-5 Horizontal Puller (Narishige Scientific Instrument Laboratory, Tokyo) from glass tubing having a 1.0 mm outside diameter and 0.5 mm internal diameter. The glass tubing contained a small glass capillary fused to the inner wall. The tips of the microelectrodes were filled with 2M KCl by immersing them vertically (tips up) in a beaker of KCl which allowed the tips to fill by capillary action. The remainder of the electrode was filled by injecting 2M KCl with a 30 gauge needle. In a few of the earlier experiments, 5M potassium acetate was used to fill the microelectrodes, and no obvious difference in intracellular responses was noted. Electrodes having tip resistances of 70-120 megohms when measured in Ringer solution were used for recording.

In approximately half of the experiments a Bioelectric microelectrode holder (Bioelectric Instruments, Farmingdale, New York) containing a chlorided silver wire was used to hold the microelectrode. This was subsequently changed to a clear plastic microelectrode holder (#EH-35, E.W. Wright, Guilford, Connecticut) which contained a sintered silver-chloride contact. Both types of holders were filled with Ringer solution, and in both cases, an identical electrode immersed in the pool of Ringer solution surrounding the mounted head served as a reference electrode. Electrical contact between the head capsule and the surrounding pool of Ringer was made with a strand of twisted Kimwipe. Leads from the electrodes went to a Mentor N-950 Intracellular Probe System (Mentor Corporation, Minneapolis, Minnesota). The d.c. potential

between the two electrodes was displayed on a Tektronix Type 502 Dual Beam Oscilloscope and was also recorded on magnetic tape by a four channel FM tape recorder (A.R. Vetter Co., Rebersburg, Pennsylvania). Tapes were later played back onto a Tektronix 5111 storage oscilloscope containing two 5A18N dual trace amplifiers and a 5B12N dual time base for analysis and for making photographic records.

Resistance Measurements

Membrane resistance changes were observed by passing trains of 30 msec depolarizing pulses (1 nanoamp or less) every 100 msec through the Mentor N-950 bridge system. The bridge balance potentiometer was adjusted to give a null reading of pulse amplitude in the dark. A downward displacement of the pulse-on portion of the oscilloscope trace represents a resistance decrease, and an upward displacement, a resistance increase. The approximate magnitude of the resistance change was calculated from Ohm's law (voltage = current X resistance). For example, with 1 nanoamp pulses a 1 mv displacement of the pulse-on portion of the trace represents a resistance change of 1 megohm. The input resistance of the cell could not be calculated because the resistance of the microelectrode often increased after penetrating tissue or a cell, and the measurements obtained with the bridge system included the resistance of the cell membrane plus the electrode.

Photostimulator

A 100W Osram quartz-iodide bulb controlled by a 8.0 amp d.c. constant current source (Model SP40-10, Deltron, Inc., North Wales, Pennsylvania) was used to illuminate the ocellus. The beam was brought to focus onto one end of a 36 inch, 3 mm diameter fiber optic bundle which was positioned 2 mm from the median ocellar lens during an experi-

ment. The maximum intensity of light at the ocellar lens, as measured with a photometer (Optometer Model 40X light meter, United Detector Technology, Santa Monica, California), was calculated to be 5.32 X 10^3 lux. This intensity of light is referred to as Log I = 0, with lower intensities (referred to as Log I = -1, etc.) produced by using Kodak Wratten neutral density filters to attenuate the beam.

Antidromic Stimulation

Two insulated metal electrodes (Transidyne General Corp.) with hooked tips were used as stimulating electrodes. They were mounted on separate manipulators and connected with shielded wires to a Grass SIU-5 stimulus isolation unit mounted inside the Faraday cage. The stimulating current was delivered from a Grass S44 stimulator. The polarity of the stimulus was reversed by a switch on the stimulus isolation unit.

The stimulating electrodes were positioned so that one electrode was hooked under the base of the ocellar nerve where it entered the brain and the other was inserted into the brain. The electrodes were then raised slightly so as to lift the ocellar nerve and the exposed portion of brain out of the pool of Ringer in order to reduce local spread of current from the stimulating electrodes to the recording electrode. Photoreceptors were impaled after the stimulating electrodes and perfusion needles were in position inside the head capsule. Although the antidromic stimulation experiments were simple in principle, they were technically quite difficult because of the small working space involved. In most experiments cells were lost because of mechanical disturbances.

Experimental Protocol

Continuous Ringer perfusion was initiated immediately following the dissection. After the fiber optic, drug perfusion needles, and recording microelectrode were positioned, the preparation was dark adapted for 5 to 60 minutes. It was not necessary to tear the sheath surrounding the ocellus in order to impale cells. Photoreceptors and second order neurons were impaled by advancing a motor driven hydraulic microdrive (Model #607WCP, David Kopf Instruments, Tujunga, California) while observing the response to an occasional dim test flash. Penetration of a cell was signaled by the appearance of an inside-negative resting potential and an appropriate light-evoked response which was opposite in polarity to the extracellular light response. Resting potentials were measured by re-zeroing the trace with a calibrated bucking voltage modification of the Mentor preamplifier and subtracting the tip potential of the microelectrode which measured to -5 mv when measured in Ringer.

In a few experiments the value of the resting potential measured by this procedure may have been somewhat inaccurate because on several occasions the tip potential changed slightly after penetrating tissue, or after prolonged recording sessions when the tip potential was measured after the microelectrode was withdrawn from the cell. However, in all experiments the resting potential was at least -40 mv because a negative d.c. shift of at least that magnitude was observed when a cell was impaled. Resting potentials are noted in each figure legend.

Following impalement of a cell, the preparation was dark adapted for 5-10 minutes and an intensity-response series was recorded. Usually three consecutive test flashes at each intensity were presented,

starting with Log I = -6 and increasing in single log unit steps to Log I = 0 and then decreasing in single log unit steps back to Log I = -6 or to the dark. Upon completion of the intensity-response series, test flashes of constant intensity, duration, and frequency were delivered.

In a few experiments the preparation was allowed to dark adapt for longer periods (up to 40 minutes) prior to drug application. The response waveforms did not appear to vary significantly following longer periods of dark adaptation. The responses reached a stable waveform after 1-2 minutes of continuous test flashing and the dark potential between responses did not vary. However, during or following drug perfusion it was noted that interflash dark potential often shifted in the hyperpolarizing direction. In some experiments the interval between flashes (5-6 seconds) was not long enough to allow the interflash dark potential to recover to the dark adapted level but I chose not to vary the stimulus conditions once drug perfusion was initiated.

For the pharmacology experiments the cell was observed for 10-20 minutes prior to the application of drugs in order to insure a stable impalement. Drug perfusion was initiated by opening and closing the ball valves attached to the tubings leading from the drug and Ringer solutions on top of the cage. This procedure minimized the possibility of mechanical disturbance to the preparation, and the drip rate could be observed without opening the door to the cage. Stimulus conditions were always maintained constant during the application of drugs and during the wash period. In most experiments intensity-response series were recorded at least once during drug application and during the wash period.

I did not perform any experiments in which the preparation was maintained in continuous darkness or in a constant background light. In addition, no systematic adaptation studies were undertaken.

RESULTS

I. INTACT OCELLAR NERVE

The Photoreceptor Response

Typical photoreceptor responses were recorded from the region of the ocellus containing the photoreceptor nuclei. When the microelectrode was positioned over the proximal half of the band of pigment cells (figure 1), responses such as those shown in figures 2 and 3 were consistently observed. This type of photoreceptor response was first described by Chappell (1970) and Chappell and Dowling (1972), and was shown to originate in the retinular cell by intracellular staining with Niagra sky blue and methyl blue.

In intact nerve preparations the dark adapted resting potential measured -40 to -52 mv and was stable. Photoreceptors responded to light with a graded depolarization whose threshold was approximately 4 log units (Log I = -4) below the maximum intensity of the light stimulus (Log I = 0). The light-evoked depolarization in response to dim or moderately bright test flashes was relatively sustained or "square" shaped, while the response to more intense stimuli showed a large transient depolarizing wave which decayed to a nearly constant level of depolarization for the duration of the test flash. If stimulus conditions remained constant, consistently stable responses could be recorded for 4 or more hours.

The single ON-spike in the photoreceptor response (figure 2), which is commonly observed in the dragonfly ocellus, was blocked by tetrodotoxin (Chappell and Dowling, 1972). However, this impulse is apparently not critical to synaptic transmission because tetrodotoxin

did not alter the response of the second order neuron in any obvious way (Chappell and Dowling, 1972). The transient hyperpolarizing wave following light OFF is a unique characteristic of the dragonfly ocellar receptor response. This OFF transient was often one of the most prominent features of the response, especially at lower light intensities (figures 2, 3, 4; Graphs I, II) or after sustained periods of illumination (figures 9-14; Graphs III, IV). However, in experiments where the microelectrode was positioned more distally (closer to the rhabdomeric end of the retinula cell), the usual OFF response was not seen (figure 4, A).

Figure 4 shows two different photoreceptor responses recorded from the same preparation under identical conditions of illumination. The recording site was confirmed by visual observation before withdrawing the microelectrode. The cell impaled in the more distal region of the ocellus showed a larger than usual depolarization during illumination and no OFF oscillation. The distally recorded responses also showed "noisy" voltage fluctuations during the light-evoked response. This noise was more prevalent at lower light intensities. In addition, small discrete depolarizing potentials were seen in the dark following the dimmer test flash. When the recording electrode was repositioned about 0.1 mm more proximally, closer to the nuclear layer and receptor terminal, the more typical light response and OFF-transient were seen.

In eight different preparations, processes believed to be receptor terminals were impaled in the synaptic region of the ocellus (figure 5). Although these processes have not yet been stained, the following criteria have been used to identify them as photoreceptor terminals:

1. These cells were always impaled either immediately before or immediately after recording from an L-neuron.

2. The position of the electrode in the synaptic region was verified by visual observation.

3. The cells showed negative dark adapted potentials (45-55 mv) and depolarizing responses to light. The light-evoked depolarization was larger than the electroretinogram, which is also positive in the synaptic region of the ocellus when recorded with the same glass microelectrode.

4. The responses showed hyperpolarizing OFF oscillations and some showed an ON-spike, characteristics which are absent in the electroretinogram.

5. Resistance measurements showed an increase in conductance during illumination (figure 8).

6. In most cases, these cells were lost after only a few minutes of recording, suggesting that the processes from which recordings were obtained were unusually small. When the cell was lost, there was a sudden positive shift in membrane potential and the waveform resumed the typical shape of the electroretinogram, indicating that the recording electrode was now extracellular.

Figure 5 shows an intensity response series from a receptor terminal in an intact nerve preparation. This cell was impaled just after recording from the second order neuron shown in figure 17, A. (The microelectrode was moved only a few microns.) There was a large ON-spike at Log I = 0 and an OFF response at lower light intensities. The sustained portion of the response was smaller than the usual photoreceptor response recorded more distally, which is consistent with the concept of decremental conduction from the receptor soma to the terminal (Burkhardt,

1962; Gwilliam, 1963, 1965; Bauman, 1968; Shaw, 1968, 1972; Jarvilehto and Zettler, 1970, 1971; Ioannides and Walcott, 1971; Chappell and Dowling, 1972; Zettler and Jarvilehto, 1972; Laughlin, 1974a; Ozawa et al., 1975; Hudspeth and Stuart, 1977; Hudspeth et al., 1977; Stuart and Oertel, 1978).

Resistance measurements indicate that the light-evoked depolarization in the photoreceptor is accompanied by an increase in conductance. Figure 6 shows the results of such measurements on the distally recorded response shown in figure 4. During illumination, the pulse-on portion of the trace is displaced downward, indicating that the input resistance has decreased. Similar but less pronounced resistance changes were also observed during a more typical photoreceptor response, recorded more proximally from the nuclear region (figure 7), and in a receptor terminal (figure 8). The light-evoked resistance decrease was largest when recording more distally.

A conductance increase during illumination has not been previously reported for dragonfly ocellar receptors and suggests, as expected, that light depolarizes the dragonfly ocellar receptor by increasing the ionic permeability across the cell membrane. A conductance increase during illumination is a common observation in many invertebrate photoreceptors (Millechia and Mauro, 1969b; Shaw, 1968, 1979; Fulpius and Bauman, 1969; Brown et al., 1970; McReynolds and Gorman, 1970; Detwiler, 1976; Gorman and McReynolds, 1978; Muijser, 1979).

Additional properties of the receptor membrane in the dragonfly ocellus were not investigated. For example, it was not possible to vary the receptor membrane potential by passing d.c. current through the recording microelectrode. Numerous attempts at such experiments were

always unsuccessful because excessive noise developed which obscured the desired changes in membrane potential.

Effect of Stimulus Conditions on the Receptor OFF Response

The waveform and magnitude of the receptor OFF response was dependent upon the conditions of illumination. In dark adapted preparations the OFF hyperpolarization was characteristically most prominent following dimmer test flashes and was less pronounced following a more intense light stimulus (Chappell and Dowling, 1972). This effect of light intensity on the OFF response can be seen in the intensity-response series shown in figures 2, 3 and Graphs I and II.

Although the receptor OFF response was normally not very pronounced following a bright test flash, it could often be enhanced by increasing the duration and/or decreasing the interval between stimuli.

Figures 9, 10 and Graph III show the response to a short (0.4 sec) and long (0.85 sec) test flash (both Log I = -1) recorded from a receptor terminal. In the dark adapted condition, the response following a short stimulus was not very distinct (figure 9) but was enhanced following a longer duration stimulus (of the same intensity). The more prolonged response was recorded from a relatively light adapted preparation. In the superimposed record (figure 9) it can be seen that the OFF response had increased in amplitude and duration but the dark potential was essentially unchanged. Sustained illumination enhanced the OFF response in this receptor terminal at every light intensity above Log I = -3 (figure 10 and Graph III).

Figure 11 shows the effect of increasing the frequency and duration of Log I = -1 test flashes on the response of a photoreceptor impaled in the nuclear region of the receptor layer. A normal-

appearing OFF response was seen after 6 minutes of continuous test flashing (figure 11, A). As the interval between test flashes was decreased or the duration was increased, the OFF response increased in amplitude and duration (figure 11, B-D). Figure 11, E, shows superimposed responses (recorded at slow and fast sweep speeds) before and after the stimulus duration was increased. Note that during illumination the sustained portion of the light response remained constant and the dark potential between flashes did not vary.

Figure 12 shows the effect of increasing the frequency or duration of a more intense light stimulus (Log I = 0) on the response of the same cell shown in the preceding figure (11). Figure 12, A, shows eight consecutive responses to I = 0 test flashes after the preparation had been dark adapted for 5 minutes. The waveform of the response to unattenuated flashes was typical of most intact nerve photoreceptors; there was a prominent ON-transient and no OFF hyperpolarization. After 13 minutes of continuous test flashing (figure 12, B) there was a reduction in the ON-transient, probably as a result of light adaptation, but otherwise the response was basically unchanged. Increasing the frequency of the stimulus (figure 12, C) caused a further reduction in the ON-transient and the small notch at OFF became slightly more prominent. The dark potential between flashes shifted about 1 mv more negative. When the duration of the stimulus was increased to 5 seconds (figure 12, D), the dark potential shifted approximately 6 mv more negative and the OFF-transient became larger. Figure 13 shows superimposed light responses before and after the duration of the bright (Log I = 0) test flash was increased. Note that the sustained portion of the light response did not vary, although the dark potential prior to light-ON

was more negative before the 5 second flash. The superimposed record in figure 13 shows superimposed oscilloscope sweeps showing both responses (400 msec, 5 sec) just before light-ON, during illumination, and following light-OFF. An enhanced OFF response can be seen following the 5 second test flash.

Photoreceptor responses from another intact nerve preparation are shown in figure 14. This preparation had been perfused with Ringer solution for almost 4 hours before these records were taken. A dark adapted intensity-response series from this cell is shown in figure 3. As the duration of an unattenuated test flash (Log I = 0) was increased from 0.4 seconds to 3 seconds (figure 14), a prominent OFF response appeared. The sustained portion of the light response did not change as the duration of the stimulus was increased and the dark potential between test flashes varied only a bit (-2 mv) after the longest (3 sec) stimulus. In the superimposed record (figure 14, bottom) showing four responses as stimulus duration was increased, it can be seen that the OFF oscillation increased in amplitude and duration in response to prolonged illumination. Graph IV shows the amplitude of the receptor OFF-transient as a function of stimulus duration.

In summary, the magnitude of the receptor OFF response seemed to depend upon stimulus conditions. In dark adapted preparations a prominent OFF hyperpolarization was normally not seen following bright test flashes under the usual stimulus conditions (0.4 sec flash every 6-8 sec). Increasing the duration of the light stimulus caused the OFF response to increase in amplitude and duration. A similar but less pronounced effect was seen when the flash duration was held constant but the interval between flashes was decreased.

Under constant stimulus conditions prolonged test flashes did not usually change the sustained level of the light response, but occasionally, long duration stimuli shifted the interflash dark potential to slightly more negative values.

The effects of stimulus duration and intensity on the receptor OFF response were not examined in detail. It is not known if duration and intensity are reciprocally related in generating a constant amplitude OFF response. The possibility that metabolic effects may contribute to the observed changes has not been eliminated and requires further investigation.

L-Neuron (Second Order) Response

All second order cells ("L-neurons", Wilson, 1978a) in the present studies were impaled in the synaptic region of the ocellus which is distal to the ocellar nerve (figure 1). This region of the ocellus contains the L-neuron dendrites, the processes of large second order neurons that are both pre- and post-synaptic to receptor terminals here, and adjacent L-neuron processes (Dowling and Chappell, 1972).

The L-neuron responds to light with a graded hyperpolarization followed by a prominent depolarizing wave at light off. The L-neuron response in the dragonfly has been described in earlier reports (Chappell, 1970; Chappell and Dowling, 1972; Klingman, 1976; Klingman and Chappell, 1978), and was shown to originate in the large second order cells by intracellular staining with procion yellow (Patterson and Chappell, 1980).

Dragonfly L-neuron responses recorded from the brain have also been identified by intracellular injection of cobalt chloride, and the waveform of such responses resembled L-neuron responses recorded more

distally in the ocellar neuropil (Mobbs et al., 1981).

Typical L-neuron responses from intact nerve preparations are shown in figures 15 and 16. The light response was more phasic than that of the photoreceptor and consisted of a rapid ON-transient which cut back to a steady state plateau for the duration of the stimulus. The dark potential measured between -40 and -55 mv and was noisier than the dark potential in the photoreceptor. Although the waveform of the L-neuron response varied somewhat in different penetrations, probably as a function of the site of the recording electrode, the response during any given impalement did not vary significantly as long as stimulus conditions remained constant (figures 18 and 19).

Resistance measurements during the L-neuron response showed an increase in conductance during illumination (figure 17), which is in agreement with earlier findings (Klingman, 1976; Klingman and Chappell, 1978). This observation supports the view that the depolarized receptor releases a neurotransmitter that hyperpolarizes the L-neuron by increasing permeability, as has been suggested for the second order neuron of several invertebrate visual systems (Shaw, 1968; Laughlin, 1974b; Stuart and Oertel, 1978; Wilson, 1978b; Zimmerman, 1978; Oertel and Stuart, 1981).

In addition to a light-induced conductance increase, I consistently observed a large prolonged increase in conductance following light OFF (figure 17). The resistance decrease associated with light OFF was often larger than the change seen during illumination. In some cells the dark potential during this period of increased conductance was only slightly depolarized or at the same level as the dark adapted value (figure 17, A). In the dragonfly, the conductance increase

associated with the L-neuron OFF response might be a consequence of changes in metabolic activity, but a similar prolonged increase in conductance following light OFF has also been described in the L-neuron of the locust median ocellus, where it was thought to represent a synaptically mediated event (Wilson, 1978b).

The waveform of the L-neuron response in the dragonfly median ocellus is similar in appearance to the second order response in many invertebrate retinas (Shaw, 1968; Scholes, 1969; Autrum et al., 1970; Jarvilehto and Zettler, 1970, 1971; Chappell and Dowling, 1972; Laughlin, 1973; Menzel, 1974; Patterson and Goodman, 1974; Ozawa et al., 1975; Erber and Sandeman, 1976; Stuart and Oertel, 1978; Oertel and Stuart, 1981; Guy et al., 1979).

Stability of Ocellar Retinal Responses in Intact Nerve Preparations

In the majority of intact nerve preparations (untreated with drugs), the waveform of the receptor and L-neuron response and the dark potential between responses did not vary as long as stimulus conditions remained constant (figures 18 and 19). However, approximately 15% of the receptors and L-neurons impaled in intact nerve preparations showed small, regular oscillations in the dark (figures 19, 2, 15). Under stimulus conditions of continuous test flashing, the oscillations appeared several seconds following light OFF, the delay apparently dependent on stimulus intensity and the interval between flashes (figure 19). This type of oscillatory activity was considered to be within the normal range of ocellar retinal responses because, except for the oscillations, the dark potential and light responses in such cells were not obviously different from those preparations showing no oscillatory activity (figures 18, 3, 16). In both cases, consistently stable

responses could be recorded for four or more hours.

Typical characteristics of receptor and L-neuron responses from intact nerve preparations can be summarized as follows: 1. Dragonfly ocellar photoreceptors respond to light with graded depolarizations followed by a hyperpolarizing OFF-transient and L-neurons respond to light with graded, more phasic, hyperpolarizations followed by a depolarizing OFF-transient; 2. The receptor OFF response is enhanced by increasing the duration of test flashes (the effect of long duration flashes was not examined in L-neurons); 3. In intact nerve preparations the dark adapted resting potential and the dark potential between test flashes did not vary as long as stimulus conditions remained constant; 4. The light-evoked responses in both types of cells were accompanied by a decrease in resistance which suggests that light increases the permeability across the photoreceptor membrane, causing the cell to depolarize, and that the photoreceptor, when depolarized, releases an inhibitory transmitter which increases the ionic permeability across the membrane of the L-neuron; 5. In addition, the L-neuron OFF response is also associated with a sustained conductance increase; 6. A small percentage of intact nerve preparations showed spontaneous oscillatory activity between flashes of intermediate intensity.

Spiking Units

On rare occasions processes showing light dependent impulse activity were recorded from the synaptic region of the ocellus in intact nerve preparations. Two classes of spiking cells were observed; those showing impulse activity during illumination (2 experiments) and those

showing impulse activity when the light stimulus was terminated (3 experiments). No spiking units were recorded from cut nerve preparations.

Figure 20 shows an intensity-response series from a unit showing light-excited impulse activity. This cell was impaled after recording from an L-neuron in a curare treated preparation. These records were taken when the preparation had been washing 7 minutes following the application of 1.4 mM curare for 45 minutes. When the cell was first penetrated, spontaneous dark impulse activity (in addition to the light-evoked spikes) was observed, but the dark impulses subsided in less than 1 minute. The firing pattern varied with stimulus intensity in a complex manner. In general, the spike frequency was highest at the beginning of each test flash. No spikes were observed in response to the dimmer stimuli (Log I = -4, I = -5). A similar pattern of spiking activity was observed in one other preparation that had been treated with eserine, but this cell was not held long enough to record an I-R series. No units showing light-excited spikes were ever recorded from an intact nerve preparation before drug application. Therefore, the possibility that the appearance of the spikes is correlated with the action of a drug cannot be eliminated. Processes showing light-excited impulse activity have also been reported in the locust median ocellus (Wilson, 1978a) and in the ocellar tract of the worker honeybee (Guy et al., 1979). The units showing impulse activity during illumination have not been identified by intracellular dye injection in any species. Extracellularly recorded light-excited spiking activity in the lateral ocellar nerve of the dragonfly was reported to disappear when the lateral ocellar nerve was severed, suggesting such impulses were efferent in origin (Kondo, 1978).

The second type of spiking unit showed hyperpolarizing light responses associated with a burst of impulses at light OFF (figure 21). The light-inhibited spiking unit shown in figure 21 was impaled in the synaptic region of the ocellus in a preparation that had not been treated with drugs. A normal appearing L-neuron response, showing no impulse activity, was impaled in the same preparation after losing the cell shown above. The waveform of the spiking unit shown in figure 21 had a smaller hyperpolarizing light response and a larger, more sustained OFF response than most L-neurons. L-neuron responses with a similar waveform but no impulse activity were also observed in several experiments (e.g. figure 17, B). It is interesting to note that the first spike following light-OFF was much larger than subsequent spikes (e.g. figure 21, Log I = 0, I = -1).

A process displaying similar light-inhibited impulse activity has been described in the dragonfly ocellus (Dowling and Chappell, 1972; Klingman, 1976) and the ocellus of the barnacle, where second order OFF responses are often associated with spike-like activity[2] (Stuart and Oertel, 1978; Oertel and Stuart, 1981). Light-inhibited spiking units have also been reported in the locust median ocellus (Wilson, 1978a) and in the ocellar nerve of the worker honeybee (Guy et al., 1979), although none of these have been identified by intracellular dye injection.[1,3]

Light-inhibited action potentials are readily recorded from the severed lateral ocellar nerve in the dragonfly (Ruck, 1961b,c; Kondo, 1978).

II. CUT OCELLAR NERVE

Intracellular responses from ocellar retinal neurons in the dragonfly (Chappell and Dowling, 1972; Klingman and Chappell, 1978; Patterson and Chappell, 1980), locust (Patterson and Goodman, 1974; Wilson, 1978a,b,c) and worker honeybee (Guy et al., 1979) have all been studied in animals with intact ocellar nerves. In these three species, L-neurons have been shown to arborize extensively in the brain (dragonfly: Kirkham et al., 1974; Chappell et al., 1978; Patterson and Chappell, 1980; locust: C.S. Goodman, 1974, 1976b; L.J. Goodman et al., 1975; worker honeybee: Pan and Goodman, 1977; Guy et al., 1979), and depolarizing OFF responses have been recorded from L-neuron processes in the brain of the dragonfly (Mobbs et al., 1981), the bee (Guy et al., 1979), and the median ocellar nerve of the locust (Wilson, 1978b). Therefore, I felt it was important to consider the possibility that electrical activity originating in the brain might be influencing ocellar retinal responses recorded more distally in the synaptic region. To investigate this possibility in the dragonfly, 18 experiments were performed on animals in which the ocellar nerve had been severed prior to recording.

All cells impaled in cut nerve preparations showed prominent OFF responses (figure 22) which were often larger and more sustained than those observed in intact nerve preparations. These findings indicate that the depolarizing OFF response in the L-neuron and the hyperpolarizing OFF response in the photoreceptor are generated more distally in the synaptic region of the ocellus and cannot be originating in the brain. However, in several respects, the behavior of L-neurons and

receptors in cut nerve preparations was often markedly different than in animals with intact ocellar nerves, especially with regard to the oscillatory character of the OFF response and the stability of the potential in the dark. These differences between intact and cut nerve preparations will be described in more detail in the following sections.

L-Neuron OFF Response

Figures 23 and 24 show L-neuron responses from two different cut nerve preparations. Every L-neuron impaled in a cut nerve preparation showed prominent OFF responses, which were often larger, more sustained, and more oscillatory than those seen in animals with intact ocellar nerves. The cell shown in figure 23 showed small discrete hyperpolarizing potentials in the dark and large oscillatory OFF responses following test flashes of moderate intensity. The light response appeared normal except that in some cases the depolarizing wave during the cut-back from ON-transient to plateau was more prominent than usual. These responses were recorded from a dark adapted preparation. During stimulus conditions of continuous test flashing, the OFF response lost its oscillatory appearance but remained large and sustained (see figure 44). The cell shown in figure 24 had a small sustained light response and a very large depolarizing OFF response which had a spike-like appearance following the brightest test flashes.

Changes in input resistance during an L-neuron response in a cut nerve preparation are shown in figure 25. The increase in conductance persisted for the duration of the OFF depolarization. The resistance decrease following light-OFF was larger than the resistance decrease during illumination. A similar prolonged conductance increase following light-OFF was shown earlier for intact nerve preparations (see figure 17).

These results suggest that the depolarizing OFF-transient in the L-neuron is generated more distally, in the synaptic region of the ocellus. The OFF depolarization and associated conductance increase cannot be originating in the brain because both events were not abolished when the second order processes were isolated from their cell bodies and extensive arborizations in the brain.

Photoreceptor OFF Response

In cut nerve preparations the hyperpolarizing OFF-transient of the photoreceptor also was not eliminated. Figure 26 shows a cut nerve photoreceptor in which the OFF response was especially prominent following dimmer test flashes. The receptor OFF response in most cut nerve preparations was more prolonged than in intact nerve preparations and was frequently followed by a period of enhanced dark oscillatory activity which was delayed or inhibited by brighter stimuli (figure 27). In addition, in cut nerve photoreceptors an OFF response was often seen following the dimmest stimuli, even though the light response during illumination was absent or barely detectable (figures 26 and 27).

In one cut nerve experiment a receptor terminal was fortuitously impaled in the synaptic region while searching for second order cells. As shown in figure 28, a prominent OFF response can be seen in this cell, even though the second order processes had been isolated from the brain.

In summary, cutting the ocellar nerve did not eliminate the OFF response in L-neurons, photoreceptors, or receptor terminals. Most cut nerve preparations showed enhanced OFF responses when compared to animals with intact ocellar nerves.

III. ANTIDROMIC STIMULATION OF THE OCELLAR NERVE

The facilitatory feedback model proposed by Klingman and Chappell (1978) suggests that the feedback synapses (Dowling and Chappell, 1972) from L-neuron dendrites onto receptor terminals are responsible for generating the hyperpolarizing OFF-transient observed in the photoreceptor response at light-OFF. If there is synaptic feedback onto receptor terminals, then antidromic stimulation of the ocellar nerve might be expected to evoke a synaptically mediated response in the photoreceptor. I was encouraged to attempt this type of experiment because the high degree of convergence of receptor terminals onto second order cells (Cajal, 1918; Dowling and Chappell, 1972) increased the probability that a feedback effect would be seen in any given photoreceptor. The major problem I encountered in performing these antidromic stimulation experiments was mechanical difficulties due to the limited space available in the dissected head capsule.

Figures 29 and 30 show an experiment in which extrinsic hyperpolarizing current was applied to the ocellar nerve while recording intracellularly from a photoreceptor. Two insulated metal wires with hooked tips were used as extracellular stimulating electrodes. One stimulating electrode was hooked under the base of the ocellar nerve where it entered the brain. The other was inserted into the brain and raised slightly with a manipulator to lift the major portion of the ocellar nerve and brain out of the pool of Ringer solution. In this way, local spread of current to the receptor terminals was minimized.

In response to the application of a brief hyperpolarizing current pulse (4 msec, 4 volt, brain electrode negative) to the ocellar nerve, a typical "OFF" transient was recorded intracellularly from the dark adapted photoreceptor (figure 29). The current-evoked response consisted of a transient hyperpolarizing wave followed by a small positive overshoot; the latency from the onset of the stimulus to the onset of the evoked hyperpolarization was 14 msec. The current stimulus was ineffective during light flashes when the L-neuron is already hyperpolarized. In the records taken at a slower sweep speed, it can be seen that every electrical stimulus evoked an OFF response, except during the period following light-OFF (figure 30). The antidromic stimulus was ineffective for up to 800 msec following light-OFF (figure 29), which approximately corresponds to the dark period during which the conductance in the L-neuron remains increased (figure 31). The inability of the current stimulus to evoke a receptor response following light-OFF suggests the increased conductance in the L-neuron during this period shunted the effectiveness of the applied current.

The hyperpolarizing transient in the photoreceptor evoked by electrical stimulation of the ocellar nerve apparently was due to synaptic feedback from second order processes onto photoreceptors. It seems unlikely that the observed effect was due to local spread of current from the stimulating electrodes because no response was seen when the recording electrode was extracellular, even though the stimulus artifact remained unchanged. Furthermore, a local current effect

would not be blocked during or after illumination when the recording electrode was inside the cell.

Due to technical problems additional studies of the response of the photoreceptor to antidromic stimulation of the ocellar nerve were not successful. It was not possible to hold receptors long enough to reverse the polarity of the current stimulus or to observe the effect of drug perfusion on the current evoked response in the receptors.

IV. CHARACTERISTICS OF OSCILLATORY BEHAVIOR

Spontaneous Fluctuations in Cut Nerve Preparations

The majority (75%) of cells impaled in cut nerve preparations showed enhanced oscillatory activity in the dark (e.g. figures 32, 33, 34, 37, 38).

Figure 32 shows an example of exaggerated fluctuations in a photoreceptor and L-neuron impaled in two different cut nerve preparations. During constant stimulus conditions (continuous test flashes of constant intensity, frequency, and duration), large spontaneous oscillations were seen between test flashes, and they seemed to persist indefinitely in the dark (the photoreceptor was observed for 6 minutes in the dark; the L-neuron, for 5 minutes -- figures 37 and 38). In the photoreceptor the oscillations consisted of rapidly rising sustained depolarizations, frequently alternating with large slow hyperpolarizations and spike-like activity. In L-neurons the oscillations consisted of transient hyperpolarizations often followed by small positive overshoots of the dark adapted potential (see figures 32, 33, 34, 37, 38). Thus, in several respects, the waveform of the oscillations in photoreceptors and L-neurons resembled the normal light response.

An intensity-response series from an L-neuron displaying enhanced oscillatory activity in the dark is shown in figure 33. The pattern of dark oscillations observed after 5 minutes of dark adaptation was the same as that shown for the Log I = -6 stimulus. The spontaneous oscillations were delayed or inhibited as stimulus intensity was increased. The response during illumination remained fairly

constant, although the OFF response often varied from test flash to test flash.

Figure 34 shows an intensity-response series from a cut nerve photoreceptor in which spontaneous dark fluctuations were especially pronounced. This cell was observed for more than 20 minutes in the dark, and the dark oscillations did not disappear during this period. In this cell the oscillations were not completely inhibited between bright test flashes; however, in most preparations bright test flashes inhibited the oscillations at the stimulus frequency shown here. Note the remarkable invariance of the light-evoked depolarization, despite the fact that the dark potential fluctuates as much as 15 mv.

Post-Illumination Hyperpolarization in Cut Nerve Photoreceptors

A consistent finding in photoreceptors from cut nerve preparations has been the appearance of a large (10-20 mv) sustained (20-30 sec) hyperpolarization following unattenuated (Log I = 0) test flashes (figures 35, 77, 26, 27). This large post-illumination hyperpolarization (PIH) was not normally observed in intact nerve preparations, even when recording for many hours (see figures 12, 14). A post-illumination hyperpolarization was usually correlated with the appearance of enhanced dark oscillatory activity (figures 77, 38, 27) but was observed in cut nerve photoreceptors even if dark oscillatory activity was not very pronounced (figures 35, 26).

Examples of a post-illumination hyperpolarization in cut nerve preparations are shown in figures 35 and 77. The spontaneous dark oscillatory activity was inhibited during the hyperpolarization and reappeared as the cell repolarized during recovery. An intensity-response series from these cells can be seen in figures 26 and 27. A post-

illumination hyperpolarization following bright light stimuli is normally observed in the photoreceptor response of the barnacle (Koike et al., 1970, 1971; Hudspeth et al., 1977; Hudspeth and Stuart, 1977; Ross and Stuart, 1978; Stuart and Oertel, 1978) and Limulus (Smith et al., 1968; Brown and Lisman, 1972) but has not been reported previously in the dragonfly ocellus.

"Bistable" Behavior in Intact and Cut Nerve Preparations

Two general classes of dark oscillations were observed in intracellular recordings from ocellar retinal neurons. The first type consisted of small regular appearing oscillations such as those shown in figures 19, 2, 15 and was seen in approximately 15% of cells impaled in intact nerve preparations (prior to the application of drugs). Such "normal" oscillatory behavior in intact nerve photoreceptors was not associated with pronounced fluctuations in dark potential or the appearance of a post-illumination hyperpolarization. The light responses, OFF oscillations, and interflash potential between stimuli were not noticeably different from cells that displayed no spontaneous oscillatory activity in the dark. The second type of dark oscillatory behavior, such as that commonly recorded from cut nerve preparations, was characterized by very pronounced dark potential fluctuations (figures 33, 34, 32, 37, 38) and is thought to represent an abnormal condition because exaggerated oscillatory activity was not usually seen in intact nerve preparations, even when recording for many hours (figures 18, 3, 16, 14). The term "bistable" will hereafter refer to the "abnormal" type of oscillatory behavior in which spontaneous fluctuations in dark

potential were especially pronounced. An unexpected finding was that during or after drug perfusion, many preparations with intact ocellar nerves began to exhibit bistable behavior (figure 39) similar to that seen in animals with severed ocellar nerves before the application of drugs. Some examples of such drug-induced oscillatory behavior are shown in figure 39 and will be described in greater detail in the following section. Enhanced dark oscillatory activity was seen in only three intact nerve preparations prior to drug treatment (figure 36), and all of these preparations had been punctured many times with the microelectrode during prolonged attempts at recording.

The large spontaneous oscillations characteristic of bistable behavior were observed in both photoreceptors and L-neurons. This condition appears to be a property of the entire preparation, not just a single cell, because when present, numerous cells impaled anywhere in the ocellus displayed a similar waveform at any given time. In those experiments in which the photoreceptor and L-neuron responses were recorded from the same preparation, oscillatory activity was seen in both types of cells (example: see figure 36).

Based upon their appearance, dark oscillations in bistable preparations can be divided into several categories, although the distinction between the different types was not always well defined.

In photoreceptors these included:

1. rapid spike-like depolarizations (figures 38, 34, 32, 77);
2. spontaneous hyperpolarizations (figures 53, 38, 33, 77);
3. large slow fluctuations in dark potential between test flashes (figures 39, 57, 79, 68, 79, 80);
4. post-illumination hyperpolarization following brightest test flash (figures 35, 77, 69, 70, 81)

5. two distinct dark equilibrium potentials (figures 57, 39, 73).

In second order neurons dark oscillations were less variable, consisting of single or paired hyperpolarizing transients often followed by small positive overshoots in dark potential (figures 32, 33, 36, 37).

In intact nerve preparations the development of a bistable condition was often observed during drug perfusion experiments. Under constant stimulus conditions (continuous test flashing of constant intensity, duration, and frequency), the interflash potential in such photoreceptors (prior to drug application) did not vary and coincided with the resting potential in the dark adapted state (figures 18, 51, 67, 79). The onset of bistable behavior was signalled by a delayed hyperpolarization several seconds following light-OFF. This delay in the appearance of the slow hyperpolarization decreased as the bistable condition became more pronounced (see figures 57, 79, 80). The fluctuations in the interflash dark potential during continuous test flashing could often become "stable" in the presence of a dim background light (figure 40) or during the application of a drug (figures 57, 53, 76). In the steady state condition (figures 39, 57, 73, 66, 79) the interflash dark potential appeared to oscillate around two distinct dark equilibrium levels. If the preparation was subsequently placed in the dark, the potential would recover to the more positive "resting" value in some cases; in others the dark potential seemed to stabilize at a more negative value (figures 39, 57, 68, 73). In such a situation, a single test flash to a dark adapted cell would initiate a transient positive shift in potential which was usually accompanied by some rapid oscillatory activity (figures 57, 39).

In cut nerve preparations bistable behavior in both photoreceptors and L-neurons was commonly observed before the application of drugs. Bistable behavior in ocellar neurons from cut nerve preparations was usually more pronounced in the dark adapted state than in intact nerve preparations (figures 37, 38, 33, 34).

In both intact and cut nerve preparations, a remarkable feature of the photoreceptor response was the invariance of the light-evoked depolarizations. This was true regardless of whether the dark potential just before light-ON was hyperpolarized, depolarized, or at some intermediate level (figures 33, 39, 57, 66, 67, 79, 80). The most reproducible characteristic of the dark potential in bistable receptors was the level that was reached following recovery from the OFF response. This more positive level of dark potential was apparently equivalent to the dark adapted "resting" potential in normal cells. It was also noted that the presence and latency of the ON-spike seemed to be a function of the dark potential prior to light-ON.

In summary, it appears that one major difference between stable and bistable photoreceptors was the tendency of the dark adapted potential to persist at more depolarized or more hyperpolarized states, respectively. Stable cells maintained a more positive dark adapted equilibrium potential while bistable cells spent a variable period of time at more negative dark adapted potentials, even though a test flash might transiently bring the potential back to the more positive level. The second major difference between stable and bistable photoreceptor responses was the appearance of a post-illumination hyperpolarization following unattenuated test flashes. A post-illumination hyperpolari-

zation was not seen in intact nerve preparations prior to drug treatment, even when subject to intense light adaptation (figures 12, 14, 69, 81, 82). It is not known whether the delayed slow hyperpolarizations following moderate intensity responses (figures 68, 57, 39, 79) are generated by the same mechanisms responsible for generating a post-illumination hyperpolarization following the brightest stimuli.

Spontaneous dark oscillatory behavior in L-neurons was observed under the same conditions that induced bistable behavior in photoreceptors (e.g. figures 32, 33, 36, 37). The dark oscillations in second order neurons always consisted of phasic hyperpolarizing transients often followed by small positive overshoots of the dark potential. The hyperpolarizations were associated with a decrease in resistance (figure 37). Unlike the oscillations in photoreceptors, the spontaneous hyperpolarizations in the second order cell were always transient in nature and never showed a sustained component. Therefore, the differences between the dark oscillations in second order neurons and photoreceptors were similar to the differences in the waveforms of their respective light responses. The dark adapted potential of second order neurons in bistable preparations did not appear to vary to the same extent as the dark potential in the photoreceptor. However, in several pharmacological studies it was noted that in response to bright flashes, the depolarizing OFF transient in the L-neuron response was followed by a sustained hyperpolarization lasting 1-5 seconds and up to 8 mv in amplitude (not shown). It is not known if the latter phenomena is correlated with the sustained dark hyperpolarizations observed in the photoreceptor, especially since L-neuron responses were not studied as extensively as those of photoreceptors.

Although the L-neuron dark potential did not seem to exhibit 2 distinct dark equilibrium potentials additional experiments are required to resolve this issue.

All types of dark oscillations varied with stimulus conditions in a very complex manner. If they were not present in the dark, they could often be induced by bright flashes, and if they were present in the dark, they were usually inhibited by bright flashes. Decreasing the interval between flashes often delayed their onset or inhibited them completely. In intact nerve preparations the "normal type" oscillations persisted for a variable period after the preparation was dark adapted but usually subsided after 1-4 minutes. Occasionally they reappeared spontaneously in such dark adapted preparations and in this case exhibited a periodic, bursting type behavior. In preparations displaying pronounced bistable behavior, the spontaneous oscillations appeared to persist indefinitely in dark adapted preparations, although it is not known whether they would have subsided eventually because no bistable cell was ever allowed to remain in the dark for more than 30 minutes.

Dark oscillatory behavior similar to that described here has been reported in rods and horizontal cells in the vertebrate retina (Norman and Pochobradsky, 1976) and in locust second order neurons (Wilson, 1978c). In both of these retinas the oscillations were induced by light when absent and inhibited by light when present.

The rhythmic pattern of spontaneous dark oscillations was not constant but seemed to depend upon stimulus conditions and on the prior light history of the cell. In some experiments, long periods of test flashing of constant intensity, duration, and frequency seemed to

synchronize the oscillations so that they appeared at regular intervals between test flashes. However, whenever the stimulus conditions were altered, the oscillatory pattern became more variable and less regular.

V. PHARMACOLOGICAL MODIFICATION OF OCELLAR RETINAL RESPONSES

Klingman and Chappell (1978) have shown that the response of second order neurons in the dragonfly median ocellus can be modified by various pharmacological agents known to interfere with synaptic transmission. The model they developed to explain their observations proposes that the L-neuron dendrites release feedback neurotransmitter onto receptor terminals when the L-neurons are depolarized in the dark. This model predicts that the same drugs which modify the response of the L-neuron may modify the response of the photoreceptor as well. The following experiments were undertaken to test this hypothesis.

Curare (d-Tubocuranine Chloride)

Curare, a nicotinic acetylcholine antagonist (Jenkinson, 1960; Koelle, 1975a,d), was shown to completely and reversibly abolish all activity in the L-neuron dendrite (Klingman, 1976; Klingman and Chappell, 1978). I was able to reproduce this effect in both intact and cut nerve preparations (figures 42, 41, 44, 45).

Figure 41 shows the effect of 0.14 mM curare on the response of an L-neuron from an intact nerve preparation. This cell displayed spontaneous dark oscillatory activity prior to drug application. The spontaneous dark hyperpolarizations were the first components of the response to be blocked and did not reappear during the wash period. A positive shift in dark potential was also observed during exposure to curare. This cell was lost after 18 minutes of washing, at which time the response had not completely recovered to its original waveform and dark potential. In most intact nerve experiments, 0.14 mM curare was

ineffective in completely blocking the L-neuron response (see figures 46, 48, 49), possibly because a sheath surrounding the ocellus and brain may impair drug diffusion (Gerschenfeld, 1973; Callec and Sattelle, 1973; Sattelle et al., 1976; Shaw, 1977, 1978; Hudspeth and Stuart, 1977; Wilson, 1978c; Heldman et al., 1979). The relatively rapid drug effect observed in this experiment may be related to the fact that the preparation had been punctured many times with the glass recording electrode, thus facilitating entry of the drug.

Figure 42 and Graphs V and VII show a different intact nerve experiment in which a higher concentration of curare (1.4 mM) was required to eliminate the L-neuron response. Although the waveform of the L-neuron response recovered during the wash period, the dark potential remained depolarized (figure 43 and Graph VI).

Curare also completely blocked the L-neuron response in cut nerve preparations, which strongly suggests the observed effect of the drug is due to its action on synapses in the ocellar plexiform layer and not at a site in the brain. The effect of 0.5 mM curare on the L-neuron response in two different cut nerve preparations is shown in figures 44 and 45. In cut nerve preparations the action of curare was quite rapid compared to intact nerve preparations, probably because interrupting the sheath surrounding the ocellar nerve permitted more rapid diffusion of the drug.

During the application of curare, the waveform of the L-neuron response changed in a characteristic manner before the response was eliminated (figures 46, 48, 49 and Graphs VIII-XIX). In those experiments in which it was possible to accurately monitor the dark potential,

the early change in waveform of the L-neuron response during the initial stages of curare perfusion was associated with a positive shift in the dark potential of the cell (figures 47, 49, 43 and Graphs VI, XI, XII, and XVII).

Figure 46 and Graphs VIII, X, XI, XII, XIV and XVI show the effect of two concentrations of curare on the L-neuron response from an intact nerve preparation. Prolonged application of the lower concentration (0.14 mM) did not block the light response, but the waveform changed in a characteristic manner. Subsequent treatment with a more concentrated solution (1.4 mM) abolished the light response in this cell, which was lost several minutes after washing was initiated when the response was just starting to recover. Superimposed responses stored over a period of 2 hours show the change in waveform was associated with a positive shift in the dark potential of the cell (figure 47), and in the intensity-response series shown in figure 48 and Graphs XIII-XVI, it can be seen that this change in waveform was especially noticeable during moderate intensity stimuli.

The change in waveform of the L-neuron response during curare perfusion can be seen more clearly in figure 49 and Graphs XVII-XIX. Initially, the dark potential became more positive while the amplitude of the ON-transient increased correspondingly. At the same time the small depolarizing wave preceding the cutback from ON-transient to plateau (normally more prominent during brighter test flashes; e.g. figures 15, 48, 16) became more positive. With continued perfusion the depolarizing OFF response and the sustained component of the light response gradually disappeared (Graphs XVII and XIX). During the wash

period, the waveform changed in the reverse order as the response recovered (figures 42, 41, 45 and Graphs V and XII).

In the experiments shown in figures 41, 42, 46 and Graphs VI, XI, XII the dark potential depolarized and persisted at a more positive level for the duration of the experiment. However, in the experiment shown in figure 49, the dark potential showed a two-phased shift, depolarizing for the first 15 minutes of curare application and then repolarizing back toward the original dark potential as perfusion continued. Graphs XVII, XVIII and XIX show the effects of curare on the response of the L-neuron.

After the second order response was abolished by curare, a long latency residual depolarization was often observed (e.g. figures 41, 42, 46). The latency and waveform of this residual depolarization were similar to the electroretinogram recorded when the microelectrode was extracellular. Figure 50 shows intracellular and extracellular records during the experiment shown in figure 42. The lower record shows the intracellular light response just before it was blocked by curare. The middle trace shows the response from the same cell one minute after the light-evoked hyperpolarization had completely disappeared. The upper trace shows the electroretinogram 20 minutes later, just after withdrawing the microelectrode from the cell. When the electrode was withdrawn, the light response had recovered to the point shown in figure 42. The transient overshoot of the dark potential during the plateau phase of the response was still present at this time.

In both intact and cut nerve preparations, the response of the photoreceptor was also modified by the application of curare.

Figure 51 and Graphs XX and XXI show the effect of 1.4 mM curare on the photoreceptor response from an intact nerve preparation. The OFF hyperpolarization decreased slightly in amplitude (Graph XX, XXI) and gradually increased in duration (Graph XXI) until it seemed to disappear. Although curare prolonged the receptor OFF response in all experiments, a large reduction in the magnitude of the OFF hyperpolarization (measured from the sustained portion of the light response) was not always observed. However, in 3 experiments the OFF response appeared to be "blocked" because the dark potential before light-ON and following light-OFF were equivalent. Consequently, it is possible that the depolarizing recovery phase of the receptor OFF transient was eliminated in such experiments.

Figure 52 and Graph XXII show this effect in a cut nerve preparation perfused with 0.5 mM curare. The OFF transient in this cell did not undershoot the dark potential prior to light on (see figure 52). During the application of curare the receptor OFF response was eliminated (see Graph XXII) and the dark potential between constant intensity test flashes shifted slightly more positive.

In 4 experiments the dark potential just prior to the test flash showed a slight (1-2 mv) depolarization. However, the effects of curare on the receptor dark potential are inconclusive because no experiments on dark adapted preparations were undertaken. Furthermore, in intact nerve preparations it is not certain whether the effects of curare such as those shown in figure 51 represent complete drug action. In intact nerve preparations the time required to observe a drug effect varied considerably.

In addition to reducing or blocking the OFF response, curare blocked the spontaneous dark oscillatory activity in a receptor from a cut nerve preparation. Figure 53 shows a cell that displayed the large amplitude dark oscillations often observed in receptors from cut nerve preparations. The bistable condition persisted for almost one hour before the application of drugs. Curare (0.5 mM) rapidly blocked the oscillations between test flashes and reduced the amplitude of the OFF response. During the wash period, the response did not recover its original waveform or oscillatory character.

There were no experiments in which the photoreceptor response completely recovered to its original waveform following curare perfusion. Instead, most preparations which had stable dark potentials prior to the application of curare developed bistable behavior during the wash period. An example of such bistable behavior in an intact nerve photoreceptor is shown in figures 39C, 40B, 57. All of these cells were treated with 1.4 mM curare. Two experiments were performed in which intact nerve preparations were treated with a lower concentration. In one experiment the OFF response changed as in figure 51 and the dark potential transiently depolarized 2 mv. This cell was lost after 4 minutes of washing after partial recovery (but no bistable behavior) was observed, and no additional cells were examined in that preparation. In the second experiment prolonged application of 0.14 mM curare slightly increased the duration of the OFF response but did not block it. The dark potential also depolarized 1-2 mv.

The effects of curare on the photoreceptor response can be summarized as follows:

1. Curare reduced or blocked the OFF response in intact and cut nerve preparations (figures 51, 52 and Graphs XX, XXI and XXII.
2. Curare blocked the spontaneous dark oscillations in photoreceptors from cut nerve preparations (figure 53).
3. Following the application of curare, several intact nerve preparations developed bistable behavior, although they had stable dark potentials prior to drug treatment (figures 57, 39C, 40B).
4. The dark potential in the photoreceptor appeared to depolarize 1-2 mv during the application of curare. This change was considered to be too small to be significant without additional experiments. Photoreceptor responses were examined in 6 intact nerve preparations and 4 cut nerve preparations during curare perfusion. L-neuron responses from 7 intact nerve preparations and 3 cut nerve preparations were examined during curare perfusion.

The effects of curare on the L-neuron response can be summarized as follows:

1. In intact nerve preparations curare reversibly blocked the hyperpolarizing light response of the L-neuron (figure 42), which agrees with the earlier findings of Klingman and Chappell (1978).
2. Curare also blocked the L-neuron response in cut nerve preparations (figures 44, 45), suggesting the observed effect is not due to drug action in the brain.
3. Spontaneous dark hyperpolarizations in L-neurons were rapidly eliminated during curare perfusion (figure 41).
4. During the application of curare, the waveform of the L-neuron response changed in a characteristic manner. Initially, the

dark potential became more positive and the ON-transient became correspondingly larger. With continued perfusion the sustained portion of the light response and depolarizing OFF response were eliminated, followed by the ON-transient (figures 46, 48, 49 and Graphs V-XIX. Frequently, after the hyperpolarizing light response of the L-neuron was eliminated, a long latency residual depolarization was observed during illumination (figures 41, 42, 46).

Cholinergic Agents

Since curare modified the OFF response in the photoreceptor and blocked all activity in the second order neuron (presumably by blocking the action of acetylcholine), it seemed that drugs which prolong or mimic the action of acetylcholine might also affect the response of ocellar retinal neurons. I tested several such agents on intact nerve preparations: 1. Eserine sulfate (physostigmine sulfate) is a drug that prolongs the action of acetylcholine by inhibiting acetylcholinesterase, the enzyme that inactivates acetylcholine (Koelle, 1975b), 2. Edrophonium chloride is a drug of simpler structure than eserine which also inhibits acetylcholinesterase but is less potent and much shorter-acting because it is more readily displaced by acetylcholine (Koelle, 1975b), 3. Carbachol (carbamylcholine chloride), a potent acetylcholine agonist, is a synthetic analog of acetylcholine that is resistant to hydrolysis by acetylcholinesterase and has a high level of nicotinic activity (Koelle, 1975a,c).

The general effect of these cholinergic agents on the response of ocellar retinal neurons appeared to mimic the action of light. Drug

perfusion experiments using cholinergic drugs were not performed on cut nerve preparations.

Photoreceptors

Eserine had a two-phased effect on the hyperpolarizing OFF response in the photoreceptor. First the OFF response became smaller and less phasic (figures 54, 56, 58). This early effect of eserine was seen in all experiments. The initial effect of eserine (reduction in the size of the OFF response) was often followed by a period in which the OFF response increased in amplitude and duration (figures 54, 56). In half of the experiments the second effect of eserine did not appear until after Ringer wash was initiated (figures 55, 58).

Figures 54 and 55 and Graphs XXIII-XXVII show the effect of two doses of eserine on the photoreceptor OFF response from an intact nerve preparation. This cell displayed small sinusoidal oscillations in the dark which are normally observed in approximately 20% of photoreceptors. At this light intensity (Log I = -1) they appeared 2 seconds following light OFF. The preparation was first treated with 0.3 uM eserine (figure 54 and Graphs XXIII and XXV) which caused the OFF response to decrease slightly in amplitude and increase slightly in duration, thus causing the OFF response to appear less phasic. With continued perfusion the OFF response increased in amplitude as well as duration, and partial recovery was seen after a 40 minute wash. During perfusion, the small sinusoidal oscillations disappeared but reappeared and increased in amplitude and duration during wash. Subsequent perfusion with 1.5 uM eserine (figure 55 and Graphs XXIV, XXVI, XXVII) caused the OFF response to change as described above, except the

effect did not reverse during wash and even continued to increase. The OFF response was more prolonged at all light intensities (Graph XXVII), and a post-illumination hyperpolarization developed after perfusion with the higher concentration (not shown).

A similar effect of eserine was seen in photoreceptors that showed no spontaneous oscillatory activity (figure 56). In the experiment shown in figure 56, a reduction in the OFF response was followed by a period in which the OFF response increased in duration. During the wash period, the duration of the OFF hyperpolarization varied from test flash to test flash. Each record shows four consecutive responses superimposed.

As mentioned earlier (pp. 41), during or after drug perfusion many photoreceptors began to exhibit large amplitude fluctuations in dark potential. Figure 57 shows a cell that had become bistable while washing after curare perfusion (later in the experiment shown in figure 51). The bistable condition developed over a period of 45 minutes following the application of curare and persisted for an additional hour before the preparation was treated with eserine. After 4 minutes of exposure to 1.5 uM eserine, the dark potential stabilized and the response appeared almost normal. The superimposed record in figure 57 shows five superimposed oscilloscope sweeps stored over a period of 12 minutes, before and during eserine perfusion. Note the presence of two distinct dark equilibrium potentials and the remarkable invariance of the light-evoked depolarization. Continued eserine perfusion (figure 58 and Graph XXVIII and XXIX) caused the OFF response in this cell to change as in figures 54, 55, 56, but dark oscillatory

activity never returned. Since antiacetylcholinesterase agents are known to antagonize the effects of curare (Koelle, 1975d), it seems possible that eserine is compensating for a bistable condition induced by curare. Such a notion is supported by the observation that another application of curare to this preparation (after the eserine treatment described above) again reduced the OFF response (figure 59), similar to that shown in figure 51. These results suggest that curare and eserine are antagonizing the action of each other on cholinoceptive sites in the ocellar plexiform layer (Koelle, 1975a,b,d).

It is interesting to compare the effects of eserine perfusion and increased illumination on the response of the photoreceptor (see pp. 20). These effects include:

1. A reduction in the size of the OFF response. A reduced OFF response was seen during the initial stages of eserine perfusion (figures 54, 56, 58) and is normally observed following a bright test flash to a dark adapted preparation (figures 2, 3, 5).
2. Enhancement of the OFF response. Prolonged eserine treatment caused the OFF response to increase in amplitude and duration (figures 54, 55, 56), as did increasing the duration and frequency of a test flash (figures 9-14).
3. Blocking dark oscillations. Spontaneous oscillations in photoreceptors were blocked during eserine perfusion (figures 54, 55) and were also inhibited by bright test flashes (figures 2, 27, 26).
4. Stabilizing influence in bistable preparations. Both eserine (figure 57) and dim background illumination (figure 40) caused bistable responses to appear "normal".

Eserine, edrophonium, and carbachol had a similar effect on the L-neuron response from intact nerve preparations; they all markedly reduced the ON-transient and the sustained components of the light response, but the depolarizing OFF response was relatively less affected and in some experiments was transiently enhanced. The change in the waveform of the L-neuron response during the application of cholinergic agents resembled that seen in the presence of a constant background light (Chappell and Dowling, 1972 and figure 60) and was very different from the waveform change observed during curare perfusion (figures 46, 49).

Figures 61 and 62 show the effect of two consecutive applications of eserine (2 uM) on the response of an L-neuron. The ON-transient and sustained portions of the light response were dramatically reduced, but the OFF response persisted, despite the fact that the dark potential had depolarized by 8 mv. During the early stages of perfusion or during the wash period, the OFF response became large, slow, and irregular from test flash to test flash (not shown). A large sustained OFF response was sometimes followed by a slow hyperpolarizing undershoot in the dark potential (not shown). These irregularities in the OFF response and dark potential preceded the depolarization associated with the reduced light response. The effect of eserine on the light-evoked depolarization was reproduced during a third perfusion to this cell (not shown), but the OFF overshoot disappeared (preceded by a transient enhancement) and did not recover with washing. A photoreceptor impaled a short time later showed a large light-evoked depolarization and no OFF response, similar to that shown in figure 73

during cobalt chloride perfusion.

The changes in the L-neuron response (not shown) during perfusion with edrophonium (50 uM) and a mixture of edrophonium (50 uM) and acetylcholine (0.5 mM) were less pronounced and more transient than the changes observed during the application of eserine. In four experiments the depolarizing OFF response became more sustained, but the light response was reduced only slightly or not at all. In two experiments there was a large reduction in the light response and a depolarization in dark potential, but this effect was transient and did not persist throughout the perfusion period. In two experiments no obvious drug effect was detected.

Carbachol is an analog of acetylcholine that is not hydrolyzed by acetylcholinesterase (Koelle, 1975c). Whereas eserine and edrophonium exert their effects by inhibiting acetylcholinesterase (thereby prolonging the action of endogenous acetylcholine), carbachol presumably mimics the action of acetylcholine by combining with nicotinic type cholinoceptive sites on the post-synaptic membrane (Koelle, 1975 a,c). Carbachol had a dramatic and highly reproducible effect on the L-neuron response, similar to that observed with eserine described in figures 61 and 62. Figure 63 and Graphs XXX-XXXVI show the change in the L-neuron response during the application of 10 mM carbachol. There was a transient enhancement in the OFF response; the ON-transient and sustained portions of the light response were reduced, and the cell depolarized 11 mv. The cell was lost after 30 minutes of washing after it recovered almost completely to its former waveform. The change in waveform of the L-neuron response during exposure to carbachol also

resembled the change in waveform in the presence of a dim background light (figure 60). A second cell in this preparation showed the same changes during two additional carbachol perfusions (figures 64, 65 and Graphs XXXVII-XLIII). Lower concentrations of carbachol (1 mM) were also effective in reducing the light response (not shown), but the changes were less pronounced and took longer to appear.

Although a sustained hyperpolarization in the L-neuron dark potential was not observed during the application of cholinergic agents, in some experiments (0.5-6.0 sec duration) oscillatory waves up to 5 mv in amplitude were noted during the early stages of perfusion or during the wash period. Occasionally the depolarizing OFF response was unusually large and sustained, but this was not seen in consecutive responses. Changes similar to these were also observed in L-neuron responses during the application of picrotoxin (see figure 72).

In two experiments photoreceptors were impaled in preparations that had been previously exposed to carbachol for prolonged periods. These photoreceptors showed no OFF responses at any light intensity (not shown).

The effects of cholinergic agents on the responses of ocellar retinal neurons can be summarized as follows:

1. Eserine caused the receptor OFF response to become smaller and less phasic. Continued perfusion caused the OFF response to increase in amplitude and duration.
2. Eserine reduced oscillatory activity and stabilized the dark potential in a bistable preparation.

3. Eserine, edrophonium, and carbachol reduced the amplitude of the L-neuron light response.
4. Cholinergic agents mimicked the action of light on the receptor and L-neuron response (see pp. 54-57 and figure 60).

Picrotoxin

Gamma-aminobutyric acid (GABA) antagonists such as bicuculline and picrotoxin (Curtis and Watkins, 1966; Krnjevic, 1973; Gerschenfeld, 1974; Johnston, 1975) were reported to reversibly modify the OFF response in the L-neuron dendrite (Klingman, 1976; Klingman and Chappell, 1978). These authors suggested that the transient enhancement of the L-neuron OFF response was due to a reduction in the amount of receptor transmitter released onto L-neuron dendrites brought about because the facilitatory action of the presumed feedback transmitter (GABA) on the receptor terminal was blocked by the action of the GABA antagonists. This idea was investigated by examining the effects of picrotoxin on the response of the photoreceptor in three intact nerve preparations and four cut nerve preparations.

Figures 66 and 67 show the effect of two concentrations of picrotoxin on a photoreceptor response from an intact nerve preparation. During perfusion with the lower dose (0.16 mM), the OFF response changed only a bit, but the potential between test flashes became less stable. During the application of the more concentrated solution (1.6 mM), the OFF response gradually increased in amplitude and duration and the interflash potential became bistable. Washing was begun after 16 minutes of exposure to the high dose when the potential

at light OFF had reached the hyperpolarized steady state shown in figures 66 and 67. The OFF response recovered almost completely after approximately 5 minutes of washing, but the bistable appearance of the dark potential between flashes persisted for more than 1 hour. The effects of 1.6 mM picrotoxin are also shown in Graphs XLIV-XLVI. Figure 68 shows the fluctuation in dark potential observed when the preparation was placed in the dark after washing 6 minutes and 30 minutes respectively. Unlike the bistable photoreceptor shown in figure 57, the dark potential did not remain at a more hyperpolarized level indefinately but depolarized to the more positive dark potential observed before the application of picrotoxin. However, it is possible that the dark potential would have hyperpolarized back to the more negative level if the cell had remained in the dark for a longer period of time (see figure 39, B). The waveform and time course of the slow hyperpolarizing oscillation resembled the post-illumination hyperpolarization observed following unattenuated test flashes.

In the picrotoxin experiment shown in figures 66 and 67, a post-illumination hyperpolarization developed during both picrotoxin applications (figures 69, 70). The post-illumination hyperpolarization disappeared during the first Ringer wash (after 0.16 mM picrotoxin, figure 69) but did not reverse during the second wash (after 1.6 mM picrotoxin; figure 70).

The effect of picrotoxin (1 mM) on the photoreceptor response from a cut nerve preparation is shown in figure 71 and Graph XLVII. The OFF response rapidly increased in amplitude and duration and recovered almost completely after a relatively brief wash. Since the

effect of picrotoxin was similar in intact and cut nerve preparations, the drug was apparently acting at the synaptic region of the ocellus and not at some site in the brain. These results indicate that the predominant effect of picrotoxin was to hyperpolarize the photoreceptor in the dark. A similar dark hyperpolarization was seen following exposure to curare (wash period only, figure 57), eserine (during and following perfusion, figures 54, 58), and during cobalt chloride treatment (figure 73).

In one experiment an L-neuron was treated with 1.6 mM picrotoxin (figure 72). The depolarizing OFF response became larger and slower, and there was a positive shift in the dark potential. The enhanced L-neuron OFF response during the application of picrotoxin may be correlated with the enhanced receptor OFF response described above (figures 66, 67, 71). A similar but more transient enhancement of the L-neuron OFF response was also observed during the application of cholinergic agents.

Cobalt Chloride

Cobalt (Co^{++}) is a divalent ion known to interfere with calcium dependent processes (Hagiwara and Takahashi, 1967; Weakly, 1973) presumably by competing with calcium ions. Cobalt chloride (1-3 mM) has been used as a synaptic blocking agent in the vertebrate retina (Cervetto and Piccolino, 1974; Dacheux and Miller, 1976; Wu and Dowling, 1979; Slaughter and Miller, 1981). In invertebrates 20-40 mM cobalt was necessary to block the release of receptor transmitter in *Hermissenda* (Alkon and Grossman, 1978), and 10 mM cobalt was only partially effective in blocking synaptic transmission in the

compound eye of the fly (Zimmerman, 1978).

Cobalt chloride was applied to six different intact nerve preparations in the present studies. In four experiments prolonged perfusion with 4 mM cobalt did not markedly alter the photoreceptor response, although some changes in the waveform of the light response were noted, especially during the wash period. In one experiment 6 mM cobalt hyperpolarized the photoreceptor and eliminated the OFF response (figure 73). This change was commonly observed in preparations that were exposed to high concentrations of drugs during prolonged recording sessions (8-18 hours, not shown).

The effect of cobalt on the L-neuron response was examined in one intact nerve preparation. Prolonged application of 4 mM cobalt partially blocked the light response of the L-neuron and caused a positive shift in the dark potential (figures 74, 75). The change in waveform of the L-neuron response during cobalt perfusion was similar to that observed with curare and showed good recovery during wash with normal Ringer. The ON-transient portion of the light response was the last component of the L-neuron response to be blocked. The cell shown in figures 74 and 75 was treated with two consecutive perfusions and washes (both perfusions with 4 mM cobalt).

Cobalt chloride also reduced or blocked the dark oscillations in photoreceptors from two different cut nerve preparations (figures 76, 77, 78). A similar blocking action on dark oscillatory activity was also observed during curare perfusion (figure 53).

Figure 76 shows the effect of 4 mM cobalt chloride on a cut nerve photoreceptor displaying large amplitude dark oscillations often

observed in such preparations. Cobalt blocked the large spontaneous depolarizations and spike-like activity between test flashes, but the sustained hyperpolarizations were not reduced and even became larger. Note that these spontaneous hyperpolarizations are distinct from the OFF response. Note also that the ON-spike, which is sensitive to tetrodotoxin (Chappell and Dowling, 1972), was not blocked by cobalt. Spontaneous dark depolarizing activity returned after a prolonged wash.

The lower record in figure 76 shows three superimposed oscilloscope sweeps stored over a period of 12 minutes, before and during cobalt perfusion. After treatment with cobalt, the dark adapted potential reached an intermediate level in between its most positive and negative value in bistable state. During the application of cobalt, the continuous depolarizing spike-like activity in the dark adapted cell was also blocked (figure 77).

The records in figure 77 were taken from the same experiment described above (figure 76; note slower sweep speed). The large sustained hyperpolarization following the brightest test flash (Log I = 0) was commonly observed in cut nerve preparations but not in animals with intact ocellar nerves before the application of drugs. In this experiment cobalt had little effect on the spontaneous dark hyperpolarizations but dramatically reduced the more rapid depolarizing oscillations.

Figure 78 shows an experiment in which prolonged application of cobalt (4 mM) to a cut nerve photoreceptor blocked (or delayed) the onset of an unusually large sustained hyperpolarization following light OFF. When this cell was impaled, the preparation had already been treated with cobalt and had been left washing under constant stimulus

conditions for more than two hours. Consequently, the unusually large "OFF" response may be related to the previous cobalt treatment and the long period of regular stimulation. A similar enhanced OFF response was never observed in intact or cut nerve photoreceptors before drug perfusion.

This photoreceptor response was subsequently observed during two consecutive cobalt applications (both 4 mM) while constant stimulus conditions were maintained. During the first cobalt treatment, a depolarizing notch appeared on the falling phase of the sustained OFF response. This depolarizing notch seemed to increase in duration until the large OFF hyperpolarization was abolished, leaving a small residual "normal" appearing OFF response. After 6 minutes perfusion the potential between test flashes appeared stable and the light response remained constant. During the wash period the reverse sequence of events occurred. A delayed hyperpolarization following light OFF appeared, and the latency of this hyperpolarization gradually decreased until it was seen as an unusually large OFF response. A similar effect was seen during the second application of cobalt to this cell, but this time after the depolarization appeared, the hyperpolarization was delayed but not eliminated. It is not possible to determine whether the sustained hyperpolarization was actually blocked during the first perfusion or whether its onset was merely delayed for a longer period of time than the interval between test flashes. As washing continued following the second perfusion, the hyperpolarizations appeared to increase in frequency until the dark oscillatory behavior characteristic of cut nerve preparations was observed.

Cobalt chloride was applied to two cut nerve preparations and six intact nerve preparations. The effects of cobalt chloride can be summarized as follows:

1. Prolonged application of cobalt (4 mM) to intact nerve preparations did not markedly alter the receptor response in preparations treated for the first time.
2. Cobalt (6 mM) hyperpolarized a receptor and eliminated the OFF response in one intact nerve preparation that had already been exposed to lengthy cobalt treatment.
3. Prolonged exposure to cobalt (4 mM) partially blocked the L-neuron response in an intact nerve preparation. (The effect of cobalt on the L-neuron response was examined in only one experiment.)
4. Cobalt chloride (4 mM) blocked the spike-like dark oscillatory activity in a cut nerve preparation but did not eliminate the large slow hyperpolarizations observed in the dark.
5. Cobalt chloride (4 mM) blocked a large slow "OFF" type hyperpolarization in a cut nerve photoreceptor that did not display any other type of oscillatory behavior when perfusion was begun. This response was examined in a preparation that had already been treated with cobalt and had been left washing for 3 hours.

The results of the cobalt experiments should be considered tentative until additional experimental evidence is available.

Ouabain

Ouabain (strophanthin-G) is an agent that inhibits the Na^{+}, K^{+}-ATPase in cell membranes (Skou, 1965). This drug was tested in

intact nerve preparations to investigate the possibility that an electrogenic sodium pump (Kerkut and Thomas, 1965; Thomas, 1972) might be contributing to the normal photoreceptor OFF response.

In six experiments 10 uM ouabain had no apparent effect on the response of the photoreceptor or L-neuron. One photoreceptor exposed to 100 uM ouabain depolarized and the light response disappeared (not shown). In one experiment the L-neuron OFF response increased in duration during the application of 10 uM ouabain (not shown). In two experiments the photoreceptor response became bistable (figures 79, 80) and developed a post-illumination hyperpolarization during prolonged ouabain perfusion (figures 81, 82). This was an unexpected finding because ouabain has been reported to block the post-illumination hyperpolarization in barnacle photoreceptors (Koike et al., 1971) and *Limulus* ventral photoreceptors (Brown and Lisman, 1972).

In summary, high concentrations of ouabain did not block the normal OFF hyperpolarization in receptors from 9 intact nerve preparations, suggesting the OFF response is not due to an electrogenic sodium pump. In two experiments the dark potential in photoreceptors hyperpolarized and the preparation developed bistable behavior and a post-illumination hyperpolarization. The effects of ouabain were not examined in either cut nerve preparations or in intact nerve preparations that already displayed bistable behavior and a post-illumination hyperpolarization.

DISCUSSION

I. SYNAPTIC ORIGIN OF OFF RESPONSES IN OCELLAR RETINAL NEURONS

The bulk of my experimental findings supports the hypothesis that OFF transients observed in intracellular responses from ocellar retinal neurons are generated by synaptic interactions in the ocellar plexiform layer (Chappell, 1970; Chappell and Dowling, 1972; Dowling and Chappell, 1972; Chappell and Klingman, 1974; Klingman, 1976; Klingman and Chappell, 1978; Wilson, 1978b,c).

Photoreceptors

An OFF response was always seen in photoreceptors impaled more proximally, nearer the nuclear layer (figures 2, 3) and receptor terminal (figure 5) but was not seen when recording more distally, closer to the rhabdomeric end of the retinula cell (figure 4). Although the exact recording sites were not identified histologically, the electrophysiological evidence suggests that the different forms of the photoreceptor response are a function of the location of the recording electrode and do not represent two classes of cells. The small depolarizing voltage fluctuations in the distally recorded response may represent the signals generated by the isomerization of visual pigment molecules due to spontaneous thermal isomerizations or the capture of single photons (Yeandle, 1958; Scholes, 1964; Kirschfield, 1966; Wu and Pak, 1975; Fuortes and O'Bryan, 1972; Lillywhite, 1977, 1978; Lillywhite and Laughlin, 1979; Shaw, 1979). Such "quantum bumps" (Yeandle, 1958), as well as the light-evoked conductance increase

(figures 6-8), should be larger when recording closer to rhabdomeric microvilli containing the photopigment and transduction apparatus (Shaw, 1979), while the OFF response would be expected to be more prominent when recording closer to the synaptic region.

It was shown that the OFF hyperpolarization in the receptor could be enhanced by increasing the duration of the test flash (Chappell and Dowling, 1972 and figures 9-14). The observation that the above phenomenon was especially pronounced when recording from the receptor terminal (figures 9, 10) suggests that the OFF response is originating in the ocellar retinal neuropil. Such an interpretation is supported by the finding that similar changes in the receptor OFF response were seen during the application of drugs known to interfere with synaptic transmission (the pharmacological results will be discussed in greater detail later).

It could be argued that the receptor OFF response is not synaptically mediated but is a consequence of the light-evoked conductance increase across the photoreceptor membrane. For example, the receptor OFF response could represent a field potential generated by extracellular currents entering receptor terminals due to a high resistance barrier in the extracellular space (Shaw, 1975, 1977, 1978, 1979; Zimmerman, 1978). Although it is possible that extracellular photocurrents might contribute to the OFF response, a field potential cannot account for all of my experimental findings because the typical receptor OFF-transient was normally more prominent at low light intensities and was present under conditions in which all of the retinula cells were receiving approximately the same level of illumination

(Chappell and Dowling, 1972 and figures 2, 3, 5), while the effect of a field potential should be more pronounced following high intensity light flashes when the receptors are not evenly illuminated (Shaw, 1975, 1979). An alternative possibility is that the receptor OFF response is activated by permeability changes during illumination. Such light dependent effects might include a voltage-dependent potassium conductance (Leonard and Lisman, 1981)[4], a delayed potassium conductance (Detwiler, 1976; Hanani and Shaw, 1977; Brown and Mote, 1974; Muijser, 1979; Coles and Tsacopoulos, 1979), or an electrogenic sodium pump (Kerkut and Thomas, 1965; Koike et al., 1971; Brown and Lisman, 1972). In the dragonfly ocellus it is unlikely that the OFF hyperpolarization in the receptor is due to an electrogenic sodium pump because the OFF response was not blocked by ouabain (figure 79, 80), a drug that inhibits the sodium-potassium ATPase (Skou, 1965).

The strongest evidence that the receptor OFF response is a synaptically mediated event is implied from the antidromic stimulation experiments. Light dependent photocurrents or metabolic processes are apparently not essential for the generation of the OFF hyperpolarization because normal appearing OFF-transients could be evoked in dark adapted photoreceptors in response to antidromic stimulation of the ocellar nerve (figures 29, 30).

In summary, the evidence presented here supports the view that the hyperpolarizing OFF response normally recorded from more proximal regions of the retinula cell layer is the result of synaptic transmission originating in the ocellar retinal neuropil. Indeed, the fact that a receptor OFF response is so readily recorded in the dragonfly

may simply be due to the relative ease with which the microelectrode can be positioned at a fortuitous site for detecting synaptic activity. The distally recorded response shown in figures 4 and 6 (which lacks an OFF oscillation) is similar in appearance to the photoreceptor response in the locust median ocellus (Patterson and Goodman, 1974). Although there is anatomical (L.J. Goodman, 1979) and electrophysiological (Wilson, 1978b,c) evidence for synaptic feedback from L-neurons onto photoreceptors in the locust ocellus, a receptor OFF response may not be observed in the locust because of "difficulties encountered in obtaining stable recordings from this species" (Wilson, 1978c). The photoreceptor response from the compound eye of a number of insects also resembles the distally recorded dragonfly ocellar receptor response and lacks a hyperpolarizing OFF-transient (Naka, 1961; Naka and Eguchi, 1962; Kirschfield, 1966; Bauman, 1968; Shaw, 1968; Scholes, 1969; Jarvilehto and Zettler, 1970, 1971; Zettler and Jarvilehto, 1972, 1973; Ioannides and Walcott, 1971; Laughlin, 1973; Fuortes, 1963). Synaptic feedback onto photoreceptors has not been reported in these species; therefore, the absence of a detectable OFF response is not unexpected. However, this question is not entirely resolved because there is some anatomical evidence for synaptic feedback from second order neurons (L2) onto photoreceptors in the compound eye of the fly (Strausfeld and Campos-Ortega, 1977). Graded depolarizing responses to light have also been recorded from photoreceptors in a number of invertebrate species (Hartline et al., 1952; MacNichol, 1956; Gwilliam, 1963; Millecchia and Mauro, 1969a; Shaw, 1972; Hudspeth and Stuart, 1977; Hudspeth and Stuart, 1977).

L-Neurons

In dragonfly L-neurons the depolarizing OFF-transient and associated conductance increases were not abolished when the second order processes were isolated from the brain by severing the ocellar nerve (figures 22, 23, 24, 25). Therefore, the L-neuron OFF response must be originating in the synaptic region of the ocellus and cannot be solely attributed to electrical activity originating in the brain.

A depolarizing OFF response in the second order neuron has been observed in several invertebrate visual systems (Shaw, 1968; Autrum et al., 1970; Jarvilehto and Zettler, 1971; Zettler and Jarvilehto, 1971; Laughlin, 1973; Menzel, 1974; Patterson and Goodman, 1974; Ozawa et al., 1975; Erber and Sandeman, 1976; Stuart and Oertel, 1978; Guy et al., 1979). Such second order responses do not normally show impulse activity, although the OFF responses often have a spike-like appearance (Wilson, 1978b), and extracellular records from the ocelli of several insects show a burst of impulses at light-OFF (Hoyle, 1955; Ruck, 1957, 1961a,b,c; Chappell and Dowling, 1972; Rosser, 1974; Kondo, 1978). In the locust median ocellus Wilson (1978b) has suggested that the "fast off transients"[5] responsible for the spike-like oscillations in some L-neuron OFF responses are the result of action potentials originating in the brain and conducted electrotonically along the ocellar nerve to the site of the recording electrode.

However, in the dragonfly the spike-like appearance of L-neuron OFF responses from cut nerve preparations (figures 22, 24) was not noticeably different from the L-neuron OFF response from animals with

intact ocellar nerves. It is not known whether the spike-like components of L-neuron OFF responses in the dragonfly correspond to the "fast off transients" described by Wilson (1978b) in the median ocellus of the locust. The origin of the spiking units shown in figures 20 and 21 also is not known.[3] Although my results do not eliminate the possibility that some regenerative properties intrinsic to the L-neuron membrane may play a role in shaping the L-neuron response (Wilson, 1978b; Guy et al., 1979), the finding that L-neuron responses were not obviously modified by tetrodotoxin (Chappell and Dowling, 1972), which blocked the extracellular impulse activity (Jeff Rind, personal communication), suggests that centrally generated action potentials do not significantly influence ocellar retinal responses recorded more distally, in the synaptic region. In summary, my electrophysiological results, in conjunction with the pharmacological data (to be discussed later), indicate that L-neuron OFF responses are generated by synaptic interactions within the ocellar plexiform layer.

II. ELECTROPHYSIOLOGICAL EVIDENCE FOR FEEDBACK

Predictions of a Sign-Conserving Feedback Model

The facilitatory feedback model first proposed by Klingman and Chappell (Chappell and Klingman, 1974; Klingman, 1976; Klingman and Chappell, 1978) leads to several predictions which can be tested experimentally. According to this model, both receptor and feedback neurotransmitters are released in the dark. The light-evoked depolarization of the retinula cell increases the amount of receptor transmitter released. Consequently, the receptor transmitter hyperpolarizes and inhibits the L-neuron dendrite and its release of feedback transmitter. The feedback transmitter has a facilitatory effect on the release of transmitter from the receptor terminals.

The following experimental findings would be expected to be a direct consequence of such a model:

1. Antidromic stimulation of the ocellar nerve should evoke a synaptically mediated response in the photoreceptor.
2. The input resistance of the L-neuron should increase when the L-neuron is hyperpolarized by intracellular current injection and decrease when the L-neuron is depolarized.
3. Depolarizing current injection into one L-neuron should evoke a hyperpolarizing response in a different L-neuron by stimulating release of feedback transmitter onto photoreceptors which, in turn, inhibits other L-neurons.
4. The L-neuron OFF response should reverse when the L-neuron is hyperpolarized by current injection.

5. Drugs that modify the response of the L-neuron should also modify the response of the photoreceptor.
6. The stability of the feedback system should be subject to perturbation.

The experimental results described in this report, in conjunction with electrophysiological studies of L-neuron responses in the locust median ocellus (Wilson, 1978b,c), are consistent with such a sign-conserving feedback model.

Antidromic Stimulation

Hyperpolarizing the ocellar nerve with extrinsic current evoked a typical "OFF" type hyperpolarization in the dark adapted photoreceptor (figures 29, 30). This current-evoked OFF response in the receptor was apparently due to synaptic feedback from L-neurons onto receptor terminals (see pp. 35-36). The most straightforward interpretation of these results is that the L-neuron releases a neurotransmitter in the dark which depolarizes the receptor terminal. Therefore, when the L-neuron processes are hyperpolarized (by current or by receptor transmitter released in response to illumination), they release less facilitatory feedback transmitter, and consequently, the receptor terminal hyperpolarizes. The observation that the electrical stimulus was ineffective during light flashes, when the second order cell is already hyperpolarized, is consistent with such an explanation.

The interpretation of the antidromic stimulation experiments is based upon the assumption that the effective current pulses were in fact hyperpolarizing the L-neuron. Unfortunately, it was not possible

to confirm this assumption by simultaneously recording from L-neurons and photoreceptors, or by monitoring the polarity of the applied current. Attempts to reverse the polarity of the extrinsic electrical stimulus invariably resulted in the loss of the receptor because the stimulus isolation unit (the Grass S44 stimulator has no reverse polarity switch) was located inside the Faraday cage. Consequently, opening the cage door or manipulating the controls on the stimulus isolation unit caused excessive mechanical disturbance. For similar reasons, attempts to modify the current evoked photoreceptor response with drugs were also unsuccessful. If the effective antidromic stimulus was depolarizing rather than hyperpolarizing L-neurons, then a sign-inverting rather than a sign-conserving feedback model is supported.

Since the antidromic stimulation experiments were performed by extrinsic stimulation of the entire median ocellar nerve, the possibility that the source of the feedback stemmed from a different class of second order (or third order) neuron cannot be ruled out. However, there is no evidence at the present time which demonstrates that the median ocellar nerve in the dragonfly contains more than one physiological class of neuron.[1]

Studies of L-Neuron Responses in the Locust

In the dragonfly, experiments designed to test the first three predictions mentioned above were inconclusive. On many occasions I attempted to pass d.c. currents through the intracellular recording electrode, but unfortunately, current greater than 1 nanoamp generated a large amount of noise (in both photoreceptors and second order cells) which obscured the desired changes in membrane potential. Apparently,

technical problems, such as these, are commonly encountered when simultaneously attempting to pass current and record from the same microelectrode (Marshall and Engberg, 1979). In the future, it should be possible to avoid these problems by employing dual recording techniques, in which intracellular responses are monitored with one microelectrode while passing current with a second microelectrode.

Martin Wilson (1978b,c) utilized dual recording techniques to study second order responses in the locust median ocellus. Although other interpretations are possible (Wilson, 1978b), the results of his elegant studies are consistent with the facilitatory feedback model (Klingman and Chappell, 1976, 1978) discussed earlier.

(A) Resistance Changes Associated with Current Injection. In the locust L-neuron Martin Wilson (1978b) studied the changes in input resistance associated with the injection of hyperpolarizing and depolarizing current. He found that hyperpolarizing the L-neuron (within the physiological range) caused the conductance of the dark adapted cell to decrease, and depolarization caused the conductance to increase. Although the author interpreted his findings in terms of voltage dependent conductance changes in the L-neuron, the results could also be explained by synaptic feedback onto photoreceptor terminals. In both cases, a non-linear current voltage relationship would be expected.

According to the facilitatory feedback model (Klingman and Chappell, 1978), hyperpolarizing the dark adapted L-neuron should reduce the amount of feedback transmitter released from this cell. Hence, the dark release of receptor transmitter should also be

reduced because the receptor terminal will be less facilitated. Therefore, the conductance across the postsynaptic membrane should decrease because the receptor transmitter apparently acts to increase the conductance of the second order cell (Shaw, 1968; Laughlin, 1974b; Klingman, 1976; Klingman and Chappell, 1978; Wilson, 1978b; Stuart and Oertel, 1978; Zimmerman, 1978). Similarly, depolarizing the L-neuron would be expected to increase the amount of feedback transmitter released which, in turn, would facilitate the dark release of receptor transmitter. Accordingly, depolarizing the L-neuron dendrite should increase the conductance in this cell.

(B) Evoking Feedback Oscillations by Depolarizing L-Neurons. In a separate study, Wilson (1978c) found that depolarizing current injected into one L-neuron evoked hyperpolarizing oscillations in a different, otherwise quiet cell. In addition, identical dark oscillatory behavior was also evoked by short flashes of light and could be produced spontaneously following the appearance of one or a few spontaneous hyperpolarizations. Because he had already demonstrated that locust L-neurons are not electrically coupled (Wilson, 1978b), he suggested that depolarizations facilitate, with a delay, synaptic transmission from receptors to second order neurons (Wilson, 1978b). This explanation is essentially the same as that proposed by Klingman and Chappell (1976, 1978) to account for their findings in the dragonfly median ocellus.

(C) Reversing the OFF Response by Hyperpolarizing Current Injection. According to the dragonfly ocellar feedback model (Chappell and Klingman, 1974; Klingman, 1976; Klingman and Chappell, 1978), in the dark an

equilibrium is established between the amount of receptor and feedback transmitter released. However, immediately following light-OFF, there is a transient period during which no receptor transmitter is released. This period when the receptor terminal is minimally facilitated probably corresponds to an early phase in the L-neuron OFF response preceding (because of synaptic delay) the subsequent effects due to the release of the feedback transmitter.

In the locust median ocellus an early depolarizing component of the L-neuron OFF response[5] was reversed by hyperpolarizing the L-neuron to membrane potentials more negative than the reversal potential for the ON-transient and sustained portions of the L-neuron light response (Wilson, 1978b). Apparently, this early depolarizing component of the L-neuron OFF response that was reversed by hyperpolarization represents a disinhibition caused by a transient cessation in the release of inhibitory neurotransmitter from the receptor terminal. Similar results would be expected in the dragonfly if the depolarizing OFF response in the L-neuron corresponds to a minimally facilitated condition in the receptor terminal in which little or no receptor transmitter is being released.

Naturally, until more electrophysiological and pharmacological data is available, caution must be exercised in applying the findings in the locust to those in the dragonfly. Nevertheless, the anatomy and fine structure of the locust median ocellus (C.S. Goodman, 1974; L.J. Goodman, Patterson, and Mobbs, 1975; L.J. Goodman, Mobbs, and Kirkham, 1979; L.J. Goodman, 1979) are similar to that of the dragonfly (Chappell and Dowling, 1972; Dowling and Chappell, 1972; Kirkham

et al., 1974; Chappell et al., 1978; Patterson and Chappell, 1980; Mobbs et al., 1981), and the L-neuron responses from both species are also similar (dragonfly: Chappell, 1972; Klingman and Chappell, 1978; locust: Patterson and Goodman, 1974; Wilson, 1978a,b,c). Therefore, it is possible that the feedback model proposed for the dragonfly (Chappell and Klingman, 1974; Klingman, 1976; Klingman and Chappell, 1978) may also apply to the locust (Wilson, 1978c).

III. LATERAL SYNAPTIC INTERACTIONS

The sign-conserving feedback model developed by Klingman and Chappell (Chappell and Klingman, 1974; Klingman, 1976; Klingman and Chappell, 1978) did not account for lateral synaptic interactions between photoreceptors and between L-neuron dendrites. Considering the fact that lateral as well as feedback synapses have been observed in the dragonfly ocellar neuropil (Dowling and Chappell, 1972), it is reasonable to assume that both play a role in shaping the response of the L-neuron.

L-Neurons

A large sustained increase in conductance was recorded from the L-neuron following the termination of a light stimulus. This prolonged conductance increase persisted for up to 800 msec following light-OFF and was seen in both intact and cut nerve preparations (figures 17, 25). The OFF conductance increase was always larger relative to the conductance increase during illumination and was present when the dark potential of the second order cell was hyperpolarized, depolarized, or at the same level as the dark potential just prior to the test flash.

One might interpret these findings in terms of the feedback hypothesis by assuming that a portion of the conductance increase was caused by an increased release of receptor transmitter which was brought about by an increased release of feedback transmitter from the L-neuron. However, since the receptor transmitter apparently acts to hyperpolarize the L-neuron dendrite, it is difficult to explain why the conductance of

the L-neuron would be increased during the slow depolarizing phase of the OFF response. The simple feedback model suggests that the OFF depolarization in the L-neuron is a consequence of reduced output of receptor transmitter. According to the model, the second order cell should remain depolarized until the released feedback transmitter has had time to increase the release of receptor transmitter and subsequently hyperpolarize the L-neuron. Clearly, a conductance increase when the L-neuron is depolarized is inconsistent with such an interpretation.

An alternative explanation to account for these observations is that the OFF conductance change in the L-neuron is due to the action of two neurotransmitters, each acting to decrease the input resistance of the L-neuron dendrite. One of the neurotransmitters could be the receptor transmitter as suggested above; the second could be the L-neuron transmitter which is released onto adjacent second order processes (as well as receptor terminals) when the L-neuron is depolarized at light-OFF. The prolonged conductance increase when the L-neuron is depolarized following light-OFF suggests that the L-neuron (feedback) transmitter tends to depolarize adjacent ocellar nerve dendrites by increasing the permeability of the membrane to an ion or ions whose equilibrium potential is close to, or more positive than, the potential of the dark adapted second order neuron. In the dragonfly median ocellus there is abundant anatomical evidence to support such a notion. Electronmicroscopic studies (Dowling and Chappell, 1972) have shown that the overwhelming majority of synapses in the ocellar plexiform layer are of the dyad type, with one presynaptic unit associated with two

postsynaptic processes. In the case where the L-neuron dendrite was the presynaptic unit, one of the postsynaptic processes was often an adjacent L-neuron dendrite, and the other, a receptor terminal. Hence, it seems probable that the L-neuron is releasing neurotransmitter onto both receptor terminals and adjacent L-neuron dendrites when the L-neuron is depolarized.

Wilson (1978b) has proposed a similar mechanism to account for the slow OFF depolarization in the L-neuron of the locust median ocellus. The L-neuron response in the locust was also associated with a large prolonged increase in conductance following light-OFF. Although Wilson did not suggest the origin of the neurotransmitters involved, he concluded that second order neurons "are postsynaptic to at least two transmitter types, one responsible for hyperpolarisation during light and one evoking depolarisation at light off". He went on to speculate that the synaptically mediated slow OFF depolarization was caused by an increase in permeability to sodium or calcium. Considering the fact that the depolarizing slow OFF-transient in the locust could not be reversed or significantly reduced by depolarizing the membrane of the L-neuron, and the observation that the conductance increase associated with light-OFF was observed when the membrane potential of the L-neuron was not depolarized, it seems doubtful that one depolarizing neurotransmitter by itself can account for the slow component of the L-neuron OFF response.

From another point of view, the evidence in the dragonfly and the locust is more consistent with the hypothesis that two neurotransmitters are simultaneously acting to increase the conductance

of the second order neuron following light-OFF. The L-neuron feedback transmitter may act to depolarize adjacent L-neuron dendrites by increasing conductance to an ion or ions with a more positive equilibrium potential (Wilson, 1978b), while the receptor transmitter (released as a consequence of feedback) would tend to hyperpolarize the L-neuron dendrite by increasing conductance to an ion or ions with a more negative equilibrium potential (Klingman and Chappell, 1978; Wilson, 1978b). The idea that the receptor transmitter increases conductance of the L-neuron to an ion with a negative equilibrium potential is supported by the observation that second order light responses from several species were reversed by hyperpolarizing the second order cell (Shaw, 1968; Laughlin, 1974a; Wilson, 1978b; Zimmerman, 1978), and the ion responsible for the hyperpolarization may be potassium (Zimmerman, 1978). Recent evidence in the compound eye of the fly indicates that second order neurons are tonically depolarized in the dark by synaptic input from cells other than photoreceptors, and the neurotransmitter at this synapse may be gamma-aminobutyric acid (Zimmerman, 1978). This also supports the idea that GABA mediated interactions between second order neurons may have a depolarizing action.

Although the resistance decrease following light-OFF suggests that lateral synaptic interactions between second order neurons might tend to depolarize L-neuron dendrites, the pharmacological evidence (to be discussed in more detail later) suggests that the equilibrium potential for the ions involved might be close to the dark adapted potential of the second order cell. This is because pharmacological

treatment with synaptic blocking agents expected to block lateral synaptic interactions between second order cells never hyperpolarized the cell, as would be predicted if such lateral synaptic interactions were depolarizing (Klingman and Chappell, 1978 and figures, 72, 74, 75).

One interesting observation was that antidromic stimulation of the ocellar nerve was ineffective in evoking a response in the photoreceptor for up to 800 msec following light-OFF (figures 29, 30). This period corresponds to the time during which the conductance in the L-neuron remained increased (figure 31). The most straightforward interpretation of these observations is that the effectiveness of the applied current might be shunted by the increased conductance in the L-neuron dendrite.

It is also possible that some metabolic process or other property intrinsic to the L-neuron membrane might contribute to the conductance increase associated with the normal L-neuron OFF response. This question was not investigated in the locust (Wilson, 1978b) and was not examined in the dragonfly. In my experiments it was not possible to accurately monitor resistance changes over long periods of time (e.g. during drug perfusion) because the resistance of the recording microelectrode often changed (usually increased) after passing current pulses for several minutes. This problem should be eliminated with dual recording techniques.

Photoreceptors

Anatomical studies have shown there are numerous lateral, reciprocal, and serial synapses between photoreceptors in the dragonfly median ocellus (Dowling and Chappell, 1972). Dyad synapses of the

receptor terminals were usually made between ocellar nerve dendrites and adjacent receptor terminals, but occasionally both postsynaptic processes were receptor terminals or both were ocellar nerve dendrites.

Lateral inhibition is thought to be one of the mechanisms important to the processing of visual information in the retina (Hartline and Ratliff, 1957; Werblin and Dowling, 1969; Werblin, 1972, 1974; Werblin and Copenhagen, 1974) and lateral synaptic inhibition between photoreceptors has been reported in the retina of Hermissenda (Alkon, 1974; Alkon and Fuortes, 1972; Heldman et al., 1979). The pharmacological evidence presented here (to be discussed later) suggests that lateral synaptic interactions between dragonfly ocellar receptors might also be inhibitory. In the fly, lateral inhibition between receptors has also been suggested to be responsible for a reduction in the hyperpolarizing response in the second order neuron when receptors of neighboring omatidia are illuminated (Zettler and Autrum, 1975; Zettler and Weiler, 1976), although the mechanism of this inhibition has not been determined.

If synaptic interactions between photoreceptors are inhibitory then lateral inhibition between receptors might contribute to the generation of the receptor OFF response. Although my experiments do not eliminate this possibility the effects of antidromic stimulation are inconsistent with such an idea. However, the interpretation of the antidromic stimulation studies (pp. 76-77) was based upon the assumptions that receptor terminals were not directly stimulated by the applied current and the effective current was, in fact, hyperpolarizing the L-neuron dendrites. If lateral inhibition between receptors

is a significant factor responsible for the receptor OFF hyperpolarization, then one would expect the receptor OFF response to be larger under conditions in which the receptors are more depolarized (i.e. bright test flashes),(see figures 2-5 and Graphs I-III). Therefore, some additional mechanism must tend to prevent the OFF-transient following brighter stimuli; perhaps the "slow process" of Lisman and Brown (1971) may play a role in this respect,[4] but see pp. 116-124.

Synaptically mediated events in the photoreceptor, due to either feedback or lateral interactions, might be expected to be associated with a change in the input resistance of the cell. However, a significant difference in resistance was not detected in the photoreceptor when comparing resistance measurements preceding and following a test flash (figures 6-8). Such a result was not surprising because lateral synaptic interactions between receptor terminals should not be very pronounced in the dark when the photoreceptor was not depolarized and might be masked by the larger conductance increase during illumination. Similarly, any synaptically mediated conductance change in the receptor terminal due to the feedback transmitter may also have been too small and/or distant from the site of the recording electrode, and the normal receptor OFF response was too brief to measure conductance changes with the bridge method. Since changes in input resistance less than one megohm were not usually detectable above the noise level of the system, small conductance changes caused by neurotransmitters need not necessarily be seen by the recording electrode.

IV. PHARMACOLOGICAL MODIFICATION OF THE RESPONSES OF OCELLAR RETINAL NEURONS

Klingman and Chappell (1978) found that the L-neuron light response can be completely and reversibly blocked by curare and that the L-neuron OFF response can be selectively abolished by GABA antagonists, such as picrotoxin and bicuculline. The facilitatory feedback model they proposed to explain their findings predicts that the same drugs that modify the response of the L-neuron dendrite should be expected to modify the response of the photoreceptor as well. Indeed, I found that curare, picrotoxin, and other agents known to interfere with synaptic transmission did modify the response of the photoreceptor, as predicted by a feedback model involving two neurotransmitters. However, the interpretation of the pharmacological results is complicated because many of the drugs tested had a two phased effect on ocellar retinal responses, suggesting that lateral synaptic interactions may also be involved.

Two phased drug effects could also be attributed to more than one class of binding site for the same transmitter, but there is no evidence at the present time to suggest multiple or novel acetylcholine or GABA receptors are present in the dragonfly.[6]

Another problem commonly encountered during pharmacological studies in invertebrates is the limited accessibility of drugs because the sheath surrounding the central nervous system sometimes acts as a barrier to diffusion. Consequently, high concentrations of drugs and/or pre-treatment of the preparation with digestive enzymes or similar

procedures are often necessary (Gerschenfeld, 1973; Callec and Sattelle, 1973; Sattelle et al., 1976; Shaw, 1977, 1978; Wilson, 1978c; Heldman et al., 1978; Hudspeth and Stuart, 1977).

The drugs employed in the experiments presented here were used as tools in an attempt to discriminate between various mechanisms involved in synaptic transmission in the ocellar retina. No systematic dose-response studies were undertaken because my main objective was to hold a cell long enough to observe an effect and reverse it with washing. The interpretation of the pharmacological data was based upon what appears to be the primary or predominant action of a given drug. It is important to recognize that the "first adage" of pharmacology is: "No drug has a single effect." (Koelle, 1975a, p. 433). Nevertheless, the drugs used in the experiments described in this report are commonly employed in such a manner (Krnjevic, 1974; Gerschenfeld, 1973), and although possible side effects must be kept in mind, there is no reason to assume (at the present time) the observed effects of these drugs were not due to their expected action, and taken cumulatively, it is important to note that the entire system can be discussed assuming conventional actions for the drugs used. Consequently, it would seem less conservative to assume that each drug action should be interpreted on the basis of non-specific effects or side effects.[6,7,8,9,10,11,12]

Curare

Curare is an acetylcholine antagonist that blocks the action of acetylcholine by reversibly combining with nicotinic acetylcholine receptors on the postsynaptic membrane[6,7,10,11,12] (Jenkinson, 1960; Koelle, 1975a,d).

In a previous study curare reversibly blocked the light response of the L-neuron in the dragonfly median ocellus (Klingman and Chappell, 1972). The fact that I was able to reproduce this effect in cut ocellar nerve preparations (figures 44, 45) as well as in intact ocellar nerve preparations (figure 42 and Graphs V-VII) strongly suggests that curare is blocking the action of acetylcholine at synapses in the ocellar retinal neuropil and not at a site in the brain. In addition, since curare also blocked the spontaneous hyperpolarizing oscillations in L-neurons (figure 41), such spontaneous hyperpolarizations are probably due to the action of acetylcholine that is released from receptor terminals in the dark.

In photoreceptors, curare reduced or blocked the OFF response in intact and cut nerve preparations (figures 51, 52 and Graphs XX-XXII) and also blocked the spontaneous dark oscillations between test flashes (figure 53). It is assumed that the period during which the OFF depolarization and spontaneous hyperpolarizations in the L-neuron were blocked corresponds to the period when the OFF response and spontaneous oscillations in the photoreceptor were also blocked, a supposition which should be confirmed in the future with simultaneous recordings from receptors and L-neurons.

The effects of curare are consistent with the hypothesis that the hyperpolarizing OFF-transient and dark oscillations in the receptor reflect synaptic feedback from the L-neuron dendrites onto receptor terminals (Chappell and Klingman, 1974; Klingman and Chappell, 1978; Klingman, 1976). Accordingly, when the L-neuron OFF response was

eliminated (figures 42, 46, 49), the receptor OFF response also disappeared (figures 51, 52 and Graphs XX-XXII).

A facilitatory feedback model could also account for the characteristic change in the waveform of the L-neuron response during the early stages of curare perfusion (figures 46, 48 and Graphs VIII-XIX). Presumably, less acetylcholine is released from receptor terminals in the dark than during illumination when the retinula cell is depolarized. Consequently, the first noticeable effect of curare might be detected as a positive shift in the dark potential of the L-neuron (figure 47 and Graphs XI, XII, XVII) because the hyperpolarizing action of acetylcholine released in the dark was blocked. Following the same reasoning, the sustained portion of the L-neuron light response should be more sensitive to curare than the ON-transient (see Graphs X, XVIII, and XIX) because the sustained portion of the light response is thought to correspond to a period in which the release of receptor transmitter is reduced (Klingman and Chappell, 1978; Wilson, 1978b). The component of the L-neuron light response that was least sensitive to the blocking action of curare was the ON-transient (see figures 46, 48 and Graphs VII, VIII, IX, XIII, XIV, XV, XVI, XVIII). The ON-transient probably represents maximal release of acetylcholine during illumination. Therefore, it was not surprising that the ON-transient might have required prolonged application and/or higher concentrations of curare before a reduction in amplitude was observed. In addition, since most curare experiments were performed using suprasaturating intensity test flashes as the light stimulus, a reduction in the hyperpolarizing action of acetylcholine at light-ON would not necessarily be apparent

if a less intense light stimulus (and less acetylcholine) also elicited a maximal ON-transient response in the L-neuron.

During the application of curare, the release of facilitatory feedback transmitter from the L-neuron in the dark might be expected to increase because the hyperpolarizing action of acetylcholine on the L-neuron should be reduced. An increase in the amount of L-neuron transmitter released could also tend to depolarize adjacent L-neuron dendrites via lateral synaptic interactions. The effects of the increased feedback might have a negligible effect on the L-neuron dark potential if the quantity of acetylcholine subsequently released was insufficient to compete with curare for the acetylcholine binding sites. Conversely, it is possible that in some experiments the dark facilitatory effects (increased quantity of acetylcholine released) might overcome the blocking action of curare, resulting in a repolarization of the L-neuron dark potential (see figure 49 and Graph XVII). Which of these opposing effects predominated could depend upon several factors, such as the accessability of the drug, the site of the recording electrode with respect to the different synaptic inputs, and the relative concentration of curare and acetylcholine at the synaptic cleft. Increased facilitation of the receptor terminal in the presence of curare might also explain why the ON-transient portion of the L-neuron light response was more resistant than the plateau to the blocking action of the drug. This is because the concentration of acetylcholine should be highest at light-ON so that competition with curare would be more effective.

In summary, the preceding discussion suggests that the positive shift in the L-neuron dark potential during curare perfusion was a postsynaptic effect due to the reduced hyperpolarizing action of acetylcholine released from receptor terminals in the dark. The large amplitude of the ON-transient would be a consequence of the positive displacement of the L-neuron membrane potential (further from the equilibrium potential of the ion responsible for the acetylcholine mediated hyperpolarization) in conjunction with the increased depolarizing lateral interactions between L-neuron dendrites. At the same time a transient negative shift in the L-neuron dark potential could be explained if the more depolarized L-neuron increased (via feedback) the amount of acetylcholine released, which could then compete more effectively with curare for binding sites on the L-neuron membrane.

A third possibility to account for the early enhancement of the L-neuron ON-transient during the initial stages of curare perfusion is that curare was blocking lateral inhibitory interactions between adjacent photoreceptors. Acetylcholine, which is thought to be the receptor transmitter, is probably released onto adjacent receptor terminals as well as L-neuron dendrites when the receptors are depolarized. If acetylcholine binding sites on receptor terminals are also sensitive to curare, then blocking lateral inhibitory interactions between receptors should increase the amount of acetylcholine released during illumination and increase the amplitude on the ON-transient response in the L-neuron. The relative contribution of lateral inhibitory interactions between photoreceptors would depend upon the site of the recording electrode, the accessibility of the drug,

and the sensitivity of cholinoceptive sites on the receptor terminal.

After the L-neuron response was blocked by curare, a small, long latency residual depolarization was often observed, especially during brighter test flashes (figures 41, 42, 46, 47 and Graph V). This residual depolarization might represent a field potential generated by a high resistance barrier in the extracellular space (Laughlin, 1974a; Shaw, 1975, 1977, 1978, 1979; Zimmerman, 1978). A high resistance barrier around the receptor terminals could block the external return path for receptor current which spreads electrotonically down the axons. The combination of high extracellular resistance and the currents produced by electrotonic depolarization of the receptor terminals at high stimulus intensities would then effectively couple the photoreceptor to the second order neuron. Thus, the intracellularly recorded L-neuron dendrite response could contain a component due to passive depolarization by extracellular currents which originate in the receptor terminals (Autrum et al., 1970; Zettler and Jarvilehto, 1971; Laughlin, 1974a; Laughlin and Hardie, 1978; Zimmerman, 1978; Shaw, 1979).

In such a situation the intracellularly recorded second order response would probably represent the sum of the true transmembrane potential and the extracellular field potential. This postulated field potential effect would be more noticeable during curare perfusion when the hyperpolarizing L-neuron response was blocked. Shaw (1975, 1979) has suggested that a delayed field potential effect could account for the reduction in the plateau portion of the L-neuron light response, either by the mechanism described above or by electrical inhibition of receptor terminals.

It should be mentioned that a high resistance barrier in the extracellular space has been studied in the insect compound eye. There is no real evidence to suggest a similar barrier exists in the ocellus, and even if present, its effects would be difficult to ascertain without an exact knowledge of the geometry of the system and the location of the low resistance shunts responsible for determining the pathway of the current.

The electrophysiological evidence is inconsistent with the hypothesis that this residual depolarization is due to the action of a curare insensitive excitatory transmitter that is normally released onto L-neurons during illumination (Wilson, 1978b).[13]

Cholinergic Agents (eserine, edrophonium, carbachol)

The effects of the application of drugs which prolong or mimic the action of acetylcholine were similar to the action of light in modifying the responses of ocellar retinal neurons. The observation that cholinergic agents and the natural light stimulus cause similar changes in the waveform of photoreceptor and L-neuron responses provides additional support for the hypothesis that the receptor transmitter is acetylcholine (Chappell and Klingman, 1974; Chappell and Kuhar, 1976; Klingman, 1976; Klingman and Chappell, 1978; Chappell, 1979).

The cholinergic agents used in this study were eserine sulfate (physostigmine sulfate), a drug that prolongs the action of acetylcholine by inhibiting acetylcholinesterase, the enzyme that inactivates acetylcholine after it is released (Koelle, 1975a,b), edrophonium chloride, a drug of simpler structure than eserine which also inhibits

acetylcholinesterase but is less potent and much shorter-acting because it is more readily displaced by acetylcholine (Koelle, 1975a,b; Blaber, 1972), a mixture of edrophonium and acetylcholine, and carbachol (carbamylcholine chloride), a synthetic analog of acetylcholine that is a potent acetylcholine agonist because it is resistant to hydrolysis by acetylcholinesterase and has a high level of nicotinic activity (Koelle, 1975a,c).

Carbachol, edrophonium, and eserine markedly reduced the amplitude of the ON-transient and plateau portion of the L-neuron light response. This change in waveform during the constant application of these drugs (figures 61-65 and Graphs XXX-XLIII) resembled the change in waveform observed in the presence of a constant background light (Chappell and Dowling, 1972 and figure 60).

Several possible mechanisms might account for the reduced L-neuron light response, assuming that side effects of the drugs such as blocking actions[10] or desensitization[9] were not responsible for the observed changes. First, prolonging or mimicking the action of acetylcholine with cholinergic agents might prolong or mimic the action of the endogenous receptor transmitter (presumably acetylcholine) on the L-neuron. Such an effect should reduce the release of a facilitatory feedback transmitter from L-neuron dendrites onto receptor terminals (Klingman and Chappell, 1978) and decrease the amount of endogenous acetylcholine released from the receptor. This could cause the L-neuron response to become smaller. Secondly, prolonging or mimicking the action of acetylcholine could enhance lateral inhibition between receptor terminals if acetylcholine released from photoreceptors onto adjacent

receptor terminals acted to inhibit its own release. Consequently, drugs that enhance lateral inhibition would cause a light flash to be less effective in evoking the release of endogenous acetylcholine from receptor terminals. Therefore, the reduced amplitude of the ON-transient and sustained components of the L-neuron light response would result from a decrease in the amount of acetylcholine released during illumination.

If eserine, edrophonium, and carbachol are prolonging or mimicking the action of acetylcholine on the receptor terminal, then by the same argument they should also enhance the inhibitory action of acetylcholine on the L-neuron. Therefore, it cannot be readily explained why the L-neuron was not observed to hyperpolarize and, in many instances, depolarized (see Graphs XXX, XXXIII, XXXIX, XL) during the application of cholinergic agents. In this regard it is interesting to note that during illumination (Chappell and Dowling, 1972; Klingman and Chappell, 1978; Wilson, 1978a,b; Guy et al., 1979) or in the presence of a constant background light (Chappell and Dowling, 1972; and figure 60), the plateau response of the L-neuron often closely approached or coincided with the dark adapted potential, even though the sustained depolarization in the photoreceptor persisted at a steady level. This suggests that the same factors that sometimes prevent the L-neuron from hyperpolarizing during sustained illumination may also be responsible for the absence of a dark hyperpolarization during treatment with cholinergic drugs. It seems possible that during sustained illumination or constant application of cholinergic drugs, lateral inhibitory inter-

actions between photoreceptors are the predominant factor responsible for the altered waveform of the L-neuron response. A secondary effect of enhanced lateral inhibition between photoreceptors could be an increase in the amount of a depolarizing neurotransmitter released from L-neurons onto adjacent L-neuron dendrites. Enhanced release of L-neuron transmitter onto adjacent L-neuron dendrites could account for the positive shift in the dark potential of the L-neuron observed during perfusion with cholinergic drugs.

In the locust ocellus (Wilson, 1978b) and the compound eye of the fly (Zimmerman, 1978), second order neurons are thought to be depolarized in the dark by a second neurotransmitter (other than the receptor transmitter). In the fly it has been suggested that this depolarizing transmitter may be GABA (Zimmerman, 1978).

Eserine (an antiacetylcholinesterase agent) appeared to have a two phased effect on the receptor OFF response. Initially the OFF hyperpolarization became smaller and less phasic (figures 54, 56, 58 and Graphs XXIII-XXV and XXVIII, XXIX), but during prolonged perfusion (or sometimes during the wash period) the OFF hyperpolarization was enhanced (figures 55, 58 and Graphs XXIV, XXVI-XXIX). Eserine also "stabilized" the dark potential of a photoreceptor that developed bistable behavior following curare perfusion (figure 57). As with L-neurons, the change in the waveform of the receptor response during the application of eserine was similar to the action of light (see figures 3, 9-14), which is consistent with the idea that the receptor transmitter may be acetylcholine because prolonging the action of endogenous acetylcholine mimicked the natural light stimulus.

These rather complex pharmacological findings are not easily interpreted in terms of a single synaptic mechanism. Similarly, it is difficult to explain why an unattenuated (Log I = 0) test flash to a dark adapted preparation does not normally evoke a prominent OFF response unless the duration of the light stimulus is increased (figures 12, 14, and Graphs III and IV). The early effect of eserine on the receptor response (reduced OFF oscillation and positive shift in the dark potential of bistable cells) suggests a depolarizing influence which may be correlated with the positive shift in the dark potential of the L-neuron observed during the application of cholinergic agents (figures 61-65 and Graphs XXX, XXXIII, XXXIX, XL). This could be explained by an increase in facilitatory feedback from L-neurons onto photoreceptors due to a reduction in the release of acetylcholine mediated by lateral inhibition between receptors. The second effect of eserine on the receptor response (increased amplitude and duration of the OFF hyperpolarization) can be explained by a delay in the release of a facilitatory transmitter from L-neuron dendrites because the inhibitory action of acetylcholine on the L-neuron was prolonged. The finding that this latter action of eserine was antagonized by curare (figure 59) and that the instability in the interflash potential following curare perfusion was blocked by eserine (figure 57) suggests that both effects depend upon the presence of acetylcholine. Curare is known to antagonize the action of cholinergic agents (Koelle, 1975b,d) and eserine is reported to antagonize the action of curare (Koelle, 1975a,b,d).

Until additional evidence is available, the simplest interpretation of the pharmacological data involving cholinergic agents is that

the acetylcholine receptors on receptor terminals are more accessible or more sensitive to the action of eserine, edrophonium, and carbachol than are the acetylcholine receptors on L-neurons. Consequently, the predominant effect of cholinergic agents was to enhance lateral inhibitory interactions between photoreceptors, while the action of endogenous acetylcholine on the L-neuron was relatively less affected. Under these circumstances, the amount of transmitter released from the L-neuron should increase and result in a depolarizing action on adjacent L-neuron dendrites and receptor terminals. This point of view is consistent with the changes in waveform observed in L-neurons and photoreceptors during the application of cholinergic agents. An alternative explanation is that the observed changes in the response of ocellar retinal neurons might be attributed to non-specific drug actions or side effects.[6,9,10,11,12]

The pharmacological results suggest that, in the dark, facilitatory feedback onto photoreceptors and depolarizing lateral synaptic interactions between L-neuron dendrites are the predominant factors responsible for the dark adapted potential in the L-neuron and photoreceptor, while during illumination, lateral inhibitory interactions between photoreceptors in conjunction with diminished facilitation of receptor terminals are more important in the shaping of the L-neuron light response. Constant exposure to cholinergic agents might shift the equilibrium in either direction. In the future, simultaneous recording from photoreceptors and L-neurons should clarify this situation.

Drug perfusion experiments using cholinergic agents have also been reported to cause biphasic changes in the responses of retinal neurons in invertebrates and vertebrates. Acetylcholine has recently been postulated to be the receptor transmitter in the nudibranch mollusc Hermissenda (Heldman and Alkon, 1978; Heldman et. al., 1979). In Hermissenda carbachol (0.1-1.0 mM) and eserine (0.1-1.0 mM) both had biphasic effects on the response of the photoreceptor. In the turtle retina acetylcholine has been suggested to be the cone to horizontal cell transmitter by a muscarinic mechanism (Gerschenfeld and Piccolino, 1977, 1979; Piccolino and Gerschenfeld, 1977). Carbachol (5 mM), eserine (2-5 mM), and edrophonium (2-5 mM) all caused biphasic effects on the horizontal cells (Gerschenfeld and Piccolino, 1979). Although the mechanisms responsible for the biphasic drug actions were not investigated in the studies cited above, it was suggested that biphasic drug effects in Hermissenda were due to drug actions on cholinergic neurons presynaptic to the receptors (Heldman et al., 1979), while biphasic drug effects on turtle cones were attributed to antagonistic drug actions due to the high concentrations (Gerschenfeld, 1979).

Lateral inhibition between photoreceptors may also be responsible for the reduced second order response in the insect compound eye when an adjacent omatidium is illuminated (Zettler and Autrum, 1975; Zettler and Weiler, 1976), but the inhibitory mechanism is not known.

Picrotoxin

Picrotoxin, an antagonist of gamma-aminobutyric acid (Curtis and Watkins, 1965; Krnjevic, 1973; Gerschenfeld, 1974; Johnston, 1978), increased the amplitude and duration of the OFF hyperpolarization in the receptor (figures 66, 67, 71 and Graphs XLIV-XLVII). The enhanced receptor OFF response during picrotoxin perfusion suggests that GABA has a depolarizing action on the receptor terminal. When this depolarization was blocked by picrotoxin, the OFF hyperpolarization became larger and more prolonged. These results are consistent with a facilitatory feedback model in which GABA is released from L-neuron dendrites and acts to depolarize the receptor terminal (Chappell and Klingman, 1974; Klingman, 1976; Klingman and Chappell, 1978).

The depolarizing OFF response in the L-neuron also became larger, more sustained, and lost its oscillatory appearance during the application of picrotoxin (Klingman, 1976; and figure 72). The enhanced OFF depolarization in the L-neuron can be explained by a reduction and/or delay in the release of acetylcholine from the receptor terminal because the depolarizing action of GABA on the receptor was blocked by picrotoxin. Following light-OFF, a reduction in the amount of acetylcholine released from more hyperpolarized photoreceptors should tend to reduce the acetylcholine mediated hyperpolarization in the L-neuron. The enhanced receptor and L-neuron OFF response during picrotoxin perfusion supports a sign-conserving feedback model utilizing GABA as the feedback transmitter (Chappell and Klingman, 1974; Klingman, 1976; Klingman and Chappell, 1978).

In an earlier section of this report it was suggested that the L-neuron transmitter (presumably GABA) tends to depolarize adjacent L-neuron dendrites via lateral synaptic interactions (see pp. 82-86). It was further suggested that GABA might increase the permeability of the L-neuron membrane to an ion or ions whose equilibrium potential is close to, or more positive than, the dark adapted equilibrium potential (pp. 83,85). The observation that the L-neuron OFF depolarization was enhanced during exposure to picrotoxin seems to indicate that picrotoxin was apparently ineffective in blocking such lateral interactions; otherwise, the L-neuron OFF response should have become smaller. On the other hand, if the L-neuron dark potential had depolarized to a point that was more positive than, or at the same level as, the equilibrium potential responsible for the laterally mediated conductance increase, then blocking such a conductance with picrotoxin would not have the effect of reducing the magnitude of the OFF depolarization and could even contribute to the enhancement. It cannot be determined whether lateral interactions between L-neurons are excitatory or inhibitory on the basis of the results presented here. Until more evidence is available, it is assumed that a laterally mediated depolarization enhances transmitter release; however, an enhanced L-neuron OFF response during the application of picrotoxin is not inconsistent with the idea that lateral synaptic interactions between L-neurons could inhibit the release of GABA from adjacent L-neuron dendrites.

It seems likely that an increase in the magnitude and duration of the L-neuron OFF depolarization would increase the amount of GABA

released from L-neurons. An increase in the amount of GABA released at light-OFF could also contribute to an enhanced OFF depolarization in the L-neuron because higher GABA concentrations should compete more effectively with picrotoxin for GABA binding sites on the L-neuron membrane. An accumulation of GABA in the dark could also account for the apparent "incomplete" blocking action of picrotoxin on the receptor terminal. For example, in most picrotoxin experiments a sustained OFF hyperpolarization in the receptor would rapidly repolarize to the normal dark potential after the preparation was placed in the dark (figures 39, 68).

An alternative explanation to account for the apparent ineffectiveness of picrotoxin in reducing a laterally mediated depolarization between L-neurons is the possibility that GABAergic sites on receptor terminals were more accessible or more sensitive to picrotoxin than were GABAergic sites on L-neuron dendrites.

In summary, the pharmacological data suggests the predominant effect of picrotoxin was to block GABA mediated feedback onto receptor terminals. It is also possible that the enhanced OFF response in the receptor was due in part to a reduction in the amount of GABA released from L-neurons if picrotoxin was also blocking lateral excitatory interactions between L-neurons. Both of these interpretations are consistent with the pharmacological studies reported by Klingman and Chappell (1978) in which picrotoxin and bicuculline blocked the depolarizing OFF overshoot in the L-neuron. However, it is recognized that additional experiments are required to confirm a GABAergic feedback model solely on the basis of the picrotoxin experiments,

especially since non-specific actions of picrotoxin have been described.[8]

The change in the waveform of the receptor OFF response during picrotoxin perfusion resembled the effects of long duration flashes in enhancing the OFF hyperpolarization (figures 9-14 and Graphs III and IV) and was also similar to the enhanced OFF hyperpolarization observed during or following prolonged exposure to eserine (figures 54, 55, 58 and Graphs XXIII-XXIX). In other words, blocking the action of GABA, sustained bright illumination, and prolonging the action of acetylcholine with eserine all tend to hyperpolarize the photoreceptor following light-OFF. These effects can be explained on the basis of feedback interactions, or lateral interactions, or both. In terms of the feedback model, picrotoxin may be enhancing the receptor OFF response by blocking the depolarizing action of GABA on the receptor terminal; sustained illumination may be enhancing the OFF response by delaying the release of GABA from L-neuron dendrites (as a consequence of increased quantities of acetylcholine released), and eserine may be enhancing the OFF response by prolonging the action of endogenous acetylcholine, leading to a delay in the release of GABA from the L-neuron. In terms of lateral synaptic interactions, picrotoxin may be reducing the amount of GABA released, by blocking excitatory synaptic interactions between adjacent L-neuron dendrites; sustained illumination may be enhancing lateral inhibition between receptor terminals (leading to a hyperpolarization in the receptor), and eserine also may have prolonged lateral inhibition between receptor terminals. Although the relative

contributions of lateral and feedback interactions are not easily distinguished, all of the evidence, taken together, is consistent with the hypothesis that the neurotransmitter released from L-neurons may be GABA (Klingman and Chappell, 1978).

Recent evidence suggests that GABA may function as a neurotransmitter in several invertebrate visual systems. Millecchia and Gwilliam (1972) reported that picrotoxin (2×10^{-4} gm/ml; approximately 0.3 mM) blocked dimming induced impulse activity in the ocellus of the rock barnacle. Zimmerman (1978) has suggested that GABA might be responsible for a synaptically mediated dark depolarization on the second order neuron in the fly compound eye, and he further postulated that the source of the GABA was not the photoreceptor.

In the vertebrate retina GABA has been postulated to be the feedback transmitter released from horizontal cells onto cones (Murakami et al., 1978; Marc et al., 1978; Lam et al., 1979a,b; Belgum and McReynolds, 1979; Wu and Dowling, 1979), and both picrotoxin and GABA induced and modified oscillatory activity in cones and horizontal cells (Negishi and Drujan, 1979). However, GABAergic neurons in the vertebrate retina may vary with the species, since recent evidence suggests that amacrine cells in the mudpuppy retina may utilize GABA as their neurotransmitter (Miller et al., 1981a,b; Frumkes et al., 1981).

<u>Cobalt Chloride</u>

Cobalt (Co^{++}) is a synaptic blocking agent that prevents the release of neurotransmitter by competing with calcium ions (Ca^{++}) at presynaptic terminals (Hagiwara and Takahashi, 1967; Weakley, 1973).

Cobalt chloride (1-3 mM) has been reported to block synaptic transmission in the vertebrate retina (Cervetto and Piccolino, 1974; Dacheux and Miller, 1976; Slaughter and Miller, 1981) and the invertebrate retina (Zimmerman, 1978; Alkon and Grossman, 1978), although higher concentrations (10-40 mM) are generally required in invertebrates.

The finding that the L-neuron response was substantially reduced by the application of cobalt (figures 74, 75) suggests that cobalt was blocking synaptic transmission between photoreceptors and L-neurons. Cobalt also blocked the receptor OFF response in an intact nerve preparation and caused the dark adapted potential to shift approximately 10 mv more negative (figure 73). The negative shift in the receptor dark potential in response to cobalt treatment can be explained if cobalt was blocking the release of a depolarizing feedback transmitter onto the receptor terminal. In addition, the observation that the receptor OFF response was blocked by cobalt further supports the hypothesis that the receptor OFF response is a synaptically mediated event (Chappell and Dowling, 1972; Dowling and Chappell, 1972) and is consistent with a sign-conserving feedback model from L-neurons onto receptor terminals (Klingman and Chappell, 1978). Photoreceptors in Hermissenda were also reported to hyperpolarize (in some experiments) during exposure to 20-40 mM cobalt (Alkon and Grossman, 1978), although the mechanism responsible for this effect was not thoroughly investigated.

Cobalt chloride also reversibly abolished the spontaneous dark oscillations in photoreceptors from cut nerve preparations (figures

76, 77, 78). In one experiment the spike-like dark oscillations but not the large slow hyperpolarizations were eliminated (figures 76, 77). In another experiment a large slow hyperpolarizing "OFF" response was blocked (figure 78). (This preparation did not display the depolarizing spike-like oscillations seen in the first experiment, possibly because of prior cobalt treatment.) The observation that similar forms of dark oscillatory activity were blocked by curare (figure 53) and eserine (figure 57) suggests that such oscillations may be dependent upon synaptic transmission. However, since the dark potential in the photoreceptors from cut nerve preparations was not observed to hyperpolarize (as was the case for the intact nerve photoreceptor), the expected action of cobalt in blocking the release of neurotransmitter may not have been complete. The latter interpretation is suggested because much of the literature seems to indicate that 4 mM Co^{++} is not sufficiently concentrated to block release of neurotransmitters in invertebrates (Alkon and Grossman, 1978; Wilson, 1978c; Zimmerman, 1978).

An alternative interpretation is that synaptic transmission was not totally responsible for the oscillatory activity in cut nerve preparations and a portion of the dark oscillations were dependent upon a calcium-dependent process intrinsic to the photoreceptor. Cobalt has been reported to block oscillatory activity in the photoreceptor of the barnacle (Ross and Stuart, 1976, 1978; Edgington and Stuart, 1979), scallop (Cornwall and Gorman, 1979), and toad rods (Fain and Quandt, 1980; Fain et al., 1977, 1980). The above workers suggested that cobalt was blocking a voltage-dependent calcium

conductance intrinsic to the photoreceptor terminal. A voltage-dependent calcium conductance could function to increase the effectiveness of small voltage changes in releasing neurotransmitter (Ross and Stuart, 1976, 1979; Egington and Stuart, 1979). However, in the barnacle it was noted that the calcium-dependent dark oscillations in receptor terminals could also have been due to synaptic input from higher order neurons onto receptor terminals (Edgington and Stuart, 1979). Recently, a calcium-dependent action potential has been identified in cones of the turtle retina where it is thought to reflect synaptic feedback from horizontal cells onto cones (Piccolino and Gerschenfeld, 1977, 1980; Gerschenfeld and Piccolino, 1979, 1980).

The cobalt sensitive oscillations in dragonfly photoreceptors may result from a voltage-dependent calcium conductance intrinsic to the receptor terminal, synaptic transmission, or both. These alternatives cannot be distinguished on the basis of the evidence presented here. A voltage-dependent calcium conductance in the receptor terminal is not inconsistent with a facilitatory feedback model because such a mechanism could enhance the effectiveness of the feedback transmitter in facilitating release of transmitter from the receptor terminal. However, the finding that cobalt, curare, and eserine changed the potential of the photoreceptor and blocked the dark oscillations suggests that at least some portion of the oscillatory activity is dependent upon synaptic transmission.

Both synaptic transmission and a voltage-dependent calcium conductance might be expected to increase the concentration of intracellular calcium. An increase in intracellular calcium concentration

at a presynaptic terminal is apparently the necessary condition required for the release of neurotransmitters (Miledi, 1973), and an increase in intracellular calcium concentration has also been reported to activate a delayed potassium conductance which causes the membrane of a neuron to hyperpolarize or oscillate (Meech, 1972, 1976; Eckhert and Lux, 1976; Gorman and Herman, 1979; Lux and Heyer, 1979; Kuba, 1980). Consequently, the effect of cobalt in transiently blocking the dark hyperpolarizations shown in figure 78 may have been an indirect effect due to blocking an increase in calcium influx.

In the dragonfly caution must be exercised in interpreting the cobalt experiments because photoreceptor responses from 4 intact nerve preparations showed only negligible effects from cobalt perfusion. A likely possibility is that a complete synaptic blocking action of cobalt was not always observed because the concentration of cobalt used in my experiments was insufficient to be completely effective in inhibiting transmitter release at presynaptic terminals. In vertebrates 1-4 mM cobalt is effective in blocking calcium-dependent processes (Cervetto and Piccolino, 1974; Dacheux and Miller, 1976; Gerschenfeld and Piccolino, 1980; Piccolino and Gerschenfeld, 1980; Slaughter and Miller, 1981) whereas 10-40 mM cobalt was required to block calcium-dependent processes in invertebrates (Ross and Stuart, 1978; Edgington and Stuart, 1979; Cornwall and Gorman, 1979; Zimmerman, 1978; Alkon and Grossman, 1978). In the median ocellus of the locust 4 mM cobalt (the concentration used in most of my experiments) was only slightly effective in blocking hyperpolarizing responses in L-neurons (Wilson, 1978c), and 10 mM Co^{++} only partially reduced the second order

response in the compound eye of the fly. Furthermore, 20-40 mM Co^{++} was required to block synaptic transmission in the visual system of Hermissenda.

Additional experiments utilizing higher concentrations of cobalt and dual-recording techniques are necessary to resolve this issue. Furthermore, the experiments reported here do not eliminate the possibility that a calcium-dependent metabolic process or novel transmitter action (Greengard, 1978; Kupferman, 1979) may be contributing to the generation of oscillatory activity.

Leonard and Lisman (1981) have suggested that an increase in intracellular calcium concentration may stabilize the light response in Limulus ventral photoreceptors by blocking a voltage-dependent potassium conductance. If a similar "slow process" (Brown and Lisman, 1971) were also present in dragonfly ocellar receptors, then cobalt might prevent such a calcium-dependent light effect and lead to hyperpolarization of the receptor membrane.

Ouabain

Nine intact nerve preparations were treated with ouabain (strophanthin-G), an inhibitor of the membrane bound sodium-potassium ATPase (Skou, 1965), to examine the possibility that the photoreceptor OFF response might be due to an electrogenic sodium pump (Kerkut and Thomas, 1965; Thomas, 1972; Carpenter, 1973). In several species a sustained hyperpolarization in the photoreceptor following intense illumination is thought to result from an electrogenic sodium pump (Smith et al., 1968; Koike et al., 1970, 1971; Brown and Lisman, 1972) which pumps more sodium out of the cell than potassium into the cell.

In six experiments prolonged application of 10 uM ouabain (the concentration used to block the PIH in the barnacle receptor) had no obvious effect on the receptor light response or the dark adapted potential, and the OFF response remained unchanged. These findings tentatively lead to the conclusion that the OFF hyperpolarization in the dragonfly ocellar receptor is not due to an electrogenic sodium pump and is not a "mini" post-illumination hyperpolarization.

In two experiments photoreceptors developed hyperpolarizing oscillations between moderate intensity test flashes (figures 79, 80) and a large sustained hyperpolarization following unattenuated stimuli (figures 81, 82) during prolonged exposure to 10 uM ouabain. These findings are inconsistent with the expected action of ouabain, which should tend to depolarize the photoreceptor by increasing intracellular sodium concentration and extracellular potassium concentration (Skou, 1965; Smith et al., 1968; Brown and Lisman, 1972). Perhaps the hyperpolarizing oscillations observed during ouabain perfusion are an indirect effect of increased intracellular sodium concentration or altered metabolic activity. For example, ouabain, by increasing intracellular sodium, is thought to promote calcium influx (Baker et al., 1969, 1971; Baker, 1972; Langer and Serena, 1970) and might lead to a hyperpolarization by activating a ouabain insensitive process (Baker, 1972; Baker et al., 1971; Meech, 1972, 1976; Eckert and Lux, 1976; Ross and Stuart, 1978; Brehm and Eckert, 1978; Wilson and Wachtel, 1978; DiPolo and Beauge, 1979; Gorman and Herman, 1979; Richardt et al., 1979; Kuba, 1980). On the other hand, it has been suggested that an increase in intracellular calcium reduces a voltage-dependent

potassium conductance in Limulus ventral photoreceptors (Lisman and Brown, 1972; Leonard and Lisman, 1981). Ouabain, by increasing intracellular sodium can stimulate a rise in intracellular calcium which leads to an attenuation of the light response (Brown and Lisman, 1972) in Limulus.

Additional reported actions of ouabain include enhancing the release of acetylcholine from the neuromuscular junction (Elmquist and Feldman, 1965) and increasing the sensitivity of the neuromuscular junction to acetylcholine (Gage, 1965). In the dragonfly ocellus the effects of ouabain on the photoreceptor response certainly merit further investigation.

Changes in Dark Potential During Drug Perfusion Experiments

In the preceding discussion of the pharmacological results it was noted that drug application often resulted in a change in the dark potential of ocellar retinal neurons. In the case of the photoreceptor response there is little doubt that such fluctuation in dark potential (usually in the hyperpolarizing direction) is a real effect because it has been shown that the level of the light evoked depolarization of the receptor response does not vary under constant conditions of illumination (e.g. see figures 32, 34, 53, 57, 66, 76 and 79). Consequently, this finding that light acts as a "current clamp" on the intracellular receptor response can help eliminate uncertainties due to random shifts in the DC level of the oscilloscope trace, which is a common problem in electrophysiological studies. However, in the case of the L-neuron response, the significance of the observed shifts in dark potential during pharmacological experiments (e.g. see figures

41-43, 46-49, 61-65, 72, 74, 75 and Graphs VI, XI, XII, XVII, XXX, XXXIII, XXXV, XXXIX, and XL) is less certain. This is because the amplitude of the L-neuron response to light was not consistently correlated with the apparent shift in the dark potential (however, see figure 49). Therefore, in some experiments, the possibility that the fluctuations in L-neuron dark potential may be artifactual (e.g. change in tip potential; change at reference electrode, etc.) cannot be eliminated on the basis of the evidence presented here. This question might be resolved by monitoring changes at the reference electrode with a separate electrode. Alternatively, the changes in L-neuron dark potential might be related to an extracellular field potential generated by currents in the extracellular space (see pp. 95-96). The factors that apparently tend to resist <u>hyperpolarizing</u> shifts in the L-neuron dark potential merit further investigation.

V. REVISED MODEL: A LOCAL CIRCUIT FEEDBACK LOOP

A revised model describing a local circuit feedback loop in the dragonfly median ocellus is shown schematically in figure 83. This new model is similar to that proposed by Klingman and Chappell (1978), except that lateral synaptic interactions between photoreceptors and L-neurons are included in addition to considering feedback interactions. This revised model is consistent with earlier anatomical (Dowling and Chappell, 1972; Patterson and Chappell, 1980), electrophysiological (Chappell and Dowling, 1972), and pharmacological (Klingman and Chappell, 1978) studies in the median ocellus of the dragonfly. In developing the model, it was necessary to make several assumptions which, at the present time, have not all been confirmed by experimental evidence. However, until proven otherwise, the following conditions are presumed to be valid:

Assumption 1

Synaptic transmission between photoreceptors and L-neurons in the dragonfly median ocellus is mediated by slow potentials, not action potentials (Chappell, 1970; Chappell and Dowling, 1972; Dowling and Chappell, 1972; Klingman and Chappell, 1978).

Comment

Signal transmission by electrotonic conduction is the primary mode of synaptic transmission in the vertebrate retina (Werblin and Dowling, 1969; Svaetichin, 1953; MacNichol and Svaetichin, 1958; Bortoff, 1964; Tomita, 1965; Tomita et al., 1967) and in many invertebrate retinas (Burkhardt, 1962; Gwilliam, 1963, 1965;

Scholes, 1969; Jarvilehto and Zettler, 1970, 1971; Chappell and Dowling, 1972; Shaw, 1968, 1972, 1979; Laughlin, 1973; Patterson and Goodman, 1974; Hudspeth and Stuart, 1977; Hudspeth et al., 1977; Stuart and Oertel, 1978; Wilson, 1978b; Guy et al., 1980).

Recent evidence indicates that voltage-dependent changes in calcium permeability may be important in modifying slow potential mediated release of transmitter within the retina (barnacle: Ross and Stuart, 1976, 1978; Stuart and Oertel, 1978; Edgington and Stuart, 1979; toad: Fain et al., 1977, 1980; Fain and Quandt, 1980; turtle: Piccolino and Gerschenfeld, 1978, 1980; Gerschenfeld and Piccolino, 1979, 1980; scallop: Cornwall and Gorman, 1979), but this still falls within the category of graded transmission since regenerative calcium spikes can usually be elicited only under non-physiological conditions.

Several workers have suggested that some hyperpolarizing responses in the retina (fly compound eye: Zettler and Jarvilehto, 1971, 1973; rods: Werblin, 1975; horizontal cells: Weiler and Zettler, 1979) and *Ascaris* muscle (Byerly and Masuda, 1979) may be regenerative; however, reports of non-decremental conduction of hyperpolarizing responses are rare, and their significance is uncertain at the present time.

Assumption 2

The photoreceptor and second order neuron each release the same functional neurotransmitter at all presynaptic sites in the same cell (see Dale, 1935).

Comment

Although several authors have proposed that some neurons might release more than one neurotransmitter (Brownstein et al., 1974; Hanley et al., 1974; Hockfelt et al., 1977; O'Donohue et al., 1982) the significance of this phenomenon is not well understood. Frequently, one or more of the additional putative neurotransmitter found in such neurons are peptides.

Assumption 3

Photoreceptors and L-neurons release more neurotransmitter when they are depolarized than when they are hyperpolarized (Hodgkin, 1972).

Comment

Despite the fact that the mechanisms responsible for slow potential mediated release of neurotransmitter are not well understood, I am not aware of any evidence that would suggest the contrary.

Assumption 4

The feedback loop in the distal ocellar retina involves only two classes of functional cell: photoreceptors and L-neuron dendrites (Cajal, 1918; Ruck and Edwards, 1954; Dowling and Chappell, 1972).

Comment

It has not been determined whether the small ocellar nerve fibers constitute a functionally distinct class of neurons.[1] The origin of the rarely recorded spiking processes has also not been established nor is it known whether such impulse activity is a normal component of the cell's response.[3]

Assumption 5

Pharmacological modification of ocellar retinal responses was due to the expected action of the drug.

Comment

In my experiments the various drugs were used as tools in an attempt to discriminate between the different components of the photoreceptor and second order responses. The drugs that I selected are commonly employed in such a manner, and no attempt was made to determine the molecular basis for their action.[6,7,8,9,10,11,12] Naturally, it is recognized that no drug has only one effect (Koelle, 1975a). Although this latter point must be kept in mind in interpreting the pharmacological results, similar use of such agents has been widely employed to study synaptic transmission in many systems (Gerschenfeld, 1973; Krnjevic, 1974).

Revised Model:

A revised model describing the feedback loop in the dragonfly median ocellus is shown schematically in figure 83. Receptor terminals and L-neuron dendrites are indicated by letters R and L,

respectively. Open arrows represent facilitatory transmitter action; black arrows, inhibitory transmitter action. The direction of the arrows point from a presynaptic process to a postsynaptic process; the number of dots represent the relative amounts of neurotransmitter released during the various stages of the light response. The responses are divided into six different phases (A-F) which are represented by the darkened portion of the intracellular responses shown to the left of each section of the diagram. These phases correspond to: (A) the dark adapted state (dark equilibrium), (B) the period immediately following light-ON (ON-transient), (C) the cutback from ON-transient to plateau, (D) the sustained (plateau) portion of the response, (E) the period immediately following light-OFF (fast OFF-transient), (F) the period extending from a short delay following light-OFF to approximately 800 msec following light-OFF (slow OFF-transient) which precedes the re-establishment of dark equilibrium condition.

This model proposes that the receptor transmitter (probably acetylcholine) is inhibitory to the L-neuron and is also inhibitory, via lateral interactions, to nearby photoreceptor terminals. The neurotransmitter released from the L-neuron (possibly GABA) provides excitatory input to GABAergic synapses on receptor terminals and also depolarizes adjacent L-neuron dendrites via lateral synapses between L-neurons.

According to the model, in the dark (A) an equilibrium is established between the amount of receptor transmitter released and the

amount of L-neuron transmitter released such that the membrane potential of the photoreceptor and L-neuron dendrite is maintained at a stable dark adapted level. Just before light-ON (A), the photoreceptor is in a relatively facilitated state compared to just after light-OFF (E), and lateral interactions between both cell types are in equilibrium. Therefore, a large amount of receptor transmitter is released at light-ON (B) when the photoreceptor depolarizes. The released acetylcholine causes a large, rapid ON-transient hyperpolarization in the L-neuron (B).

At some time following light-ON (C) (corresponding to the period represented by the cutback from ON-transient to plateau in the L-neuron), there is a decrease in the amount of neurotransmitter released from the receptor terminal. Two factors can contribute to a decreased release in receptor transmission: 1) The hyperpolarized L-neuron now releases little or no excitatory feedback transmitter onto receptor terminals. Consequently, less receptor transmitter will be released during this period (C), 2) The large amount of receptor transmitter released at light-ON (B) would subsequently tend to reduce further output of acetylcholine because of delayed lateral inhibition between receptor terminals. The relative contribution of lateral and feedback interaction in determining the amount of receptor transmitter released is difficult to ascertain because lateral and feedback interactions would be expected to be mutually interdependent. Most of the evidence suggests that lateral inhibition between receptors predominates during illumination, especially at high light intensities.

It seems probable that the transition period from ON-transient to plateau (C) in the L-neuron may result from interplay between lateral and feedback synaptic interactions. During this transition period (C) the potential in the second order neurons is sometimes observed to oscillate around the dark adapted potential (figures 15, 16, 22, 23). During the cutback from ON-transient to plateau (C) changes in the amount of L-neuron transmitter released onto adjacent L-neuron dendrites might also contribute to this transient oscillatory condition. Similar changes in the receptor terminal would be masked during illumination when the receptor terminal is depolarized.

Eventually (approximately 200 msec after light-ON) a new equilibrium condition is established which corresponds to the sustained (plateau) portion of the light response (D). Again, several factors could contribute to the magnitude of the plateau portion of the L-neuron light response. The amount of receptor transmitter released would depend upon the light intensity and the relative contribution of lateral inhibition between photoreceptors and facilitatory feedback from L-neurons. At the same time a decrease in lateral excitatory synaptic interactions between adjacent L-neuron dendrites would tend to hyperpolarize the L-neuron during illumination. Earlier, it was suggested that the L-neuron transmitter (GABA) may act to depolarize adjacent L-neuron dendrites by increasing conductance to an ion or ions whose equilibrium potential is close to or more positive than the dark adapted equilibrium potential (see pp. 82-86). Consequently, during illumination the hyperpolarized L-neuron would release less depolarizing transmitter onto adjacent L-neuron dendrites. This

would have the effect of hyperpolarizing the L-neuron by decreasing conductance to an ion with a more positive equilibrium potential. Although in some experiments the conductance decrease during the plateau phase of the L-neuron was not very large, the majority of cells showed a significant conductance increase during illumination, suggesting that the action of the receptor transmitter is the major factor responsible for the sustained hyperpolarization during illumination. It seems likely that the variation in waveform between different L-neurons during the sustained portion of the light response (D) depends upon the site of the recording electrode with respect to the different synaptic inputs and the geometry of the L-neuron. The new equilibrium condition in (D) should be especially sensitive to pharmacological manipulation and stimulus conditions because four types of synaptic interactions may simultaneously be contributing to the plateau portion of the response:

1) Inhibitory synaptic interactions from receptors to L-neurons,
2) Inhibitory synaptic interactions between adjacent receptor terminals,
3) Excitatory feedback interactions from L-neuron dendrites onto receptor terminals,
4) Depolarizing lateral interactions between adjacent L-neuron dendrites.

At light-OFF (E) the photoreceptor repolarizes, causing the release of receptor transmitter to cease. The potential in the photoreceptor undershoots the dark adapted potential because it is less facilitated during the sustained portion of the light response (D)

than in the dark adapted state (A). In the absence of receptor transmitter the L-neuron depolarizes (E) and releases a large amount of GABA onto receptor terminals and adjacent L-neuron dendrites (F).

The released L-neuron transmitter has several effects which can be seen for a variable period following light-OFF (F): 1) The L-neuron transmitter facilitates the release of receptor transmitter from the receptor terminal. This would tend to hyperpolarize the L-neuron by increasing conductance to an ion or ions whose equilibrium potential is more negative than the dark adapted potential, 2) During the same period (F) the L-neuron transmitter probably tends to depolarize the L-neuron dendrite via lateral synaptic interactions. The L-neuron transmitter (GABA) may act to increase the permeability of the L-neuron dendrite to an ion or ions whose equilibrium potential is more positive than or at the same level as the dark adapted membrane potential. Both of these effects could account for the oscillatory appearance of the L-neuron OFF response as well as the large conductance increase in the L-neuron following light-OFF (figures 17, 25). Lateral synaptic interactions between L-neurons might, by increasing conductance, also tend to stabilize the membrane potential in L-neurons so that the dark adapted equilibrium condition (A) can be re-established more quickly.

VI. PERTURBATION OF FEEDBACK LOOP

Oscillatory Behavior

In dealing with the complex and interdependent synaptic interactions in the dragonfly ocellar retina, the response waveform of the photoreceptor and second order neuron at any given moment will reflect the relative contribution of each type of synaptic interaction with respect to the others. The activation or inactivation of one portion of the loop (represented in figure 83) must necessarily influence other portions of the loop after an appropriate delay. Therefore, one is faced with an oscillating system that is constantly striving to re-establish equilibrium. Such a system would respond rapidly to transient changes in stimulus conditions, but its stability might be readily perturbed, as suggested by the experimental findings presented here. The major problem I encountered in interpreting my data was that the relative contributions of lateral and feedback interactions were difficult to ascertain. Nevertheless, my results indicate that the stability of the feedback system is disrupted by pharmacological manipulation or by isolating the second order neurons from the brain.

The enhanced dark oscillatory behavior characteristic of bistable cells was always observed in both types of ocellar retinal neurons under the same experimental conditions. Dark oscillations were seen in most cut nerve preparations before the application of drugs and in many intact nerve preparations during or after perfusion with curare, cholinergic agents, picrotoxin, and cobalt. Once present, spontaneous

oscillations could be modified by the agents mentioned above, which generally modified the normal OFF responses in preparations with stable or bistable dark adapted potentials. These findings suggest that OFF responses and dark oscillations are synaptically mediated because all of the above drugs are known to interfere with synaptic transmission. Furthermore, it cannot be entirely coincidental that synaptic blocking agents and light both evoke dark oscillations when absent, inhibit them when present, and have a similar effect on the OFF response in the photoreceptor.

In cut nerve preparations the prominent dark oscillations in photoreceptors must, in some way, be dependent upon similar dark oscillations in L-neurons. The enhanced dark oscillatory activity in cut nerve photoreceptors cannot be a direct consequence of trauma involved in cutting the nerve because photoreceptor terminals were not observed to enter the nerve (Ruck and Edwards, 1964; Patterson and Chappell, 1980). It is thought that cutting the ocellar nerve induces a bistable condition in L-neurons which, in turn, feedback onto receptor terminals, causing a bistable condition in the photoreceptor response. However, it is not clear why severing the ocellar nerve should induce such oscillatory behavior in L-neurons. It is possible that isolating the L-neuron processes from their cell bodies and extensive arborizations in the brain eliminates a large sink for current which would normally flow from the distal retinal neuropil to the brain. Such alteration of the cable properties of L-neurons would cause voltage changes to be less attenuated (Rall, 1959; Jack et al., 1975; pp. 62-72) and could lead to an instability in

the feedback loop. For example, in a cable that is one space constant in length, the voltage measured at the end of the cable should be double the voltage in an infinite cable measured at the same distance from the voltage source (Jack et al., 1975; pp. 59-82).

An alternative explanation to account for oscillatory behavior in cut nerve preparations is that severing the ocellar nerve alters the metabolic activity, or ionic environment surrounding photoreceptors, or in some way accelerates the deterioration of the preparation. According to this point of view, oscillatory behavior might be originating in deteriorating photoreceptors and be transmitted to L-neurons by synaptic interactions.

Ruck (1961b,c) has described spontaneous dark oscillatory activity recorded extracellularly from the severed end of the lateral ocellar nerve in the dragonfly. He suggested that the arrhythmic pattern of the spontaneous dark oscillations becomes rhythmic as the preparation deteriorates because spontaneous activity of the receptor cells becomes synchronous with physiological decline (Ruck, 1961b). However, I found that the rhythmic pattern of spontaneous oscillations depended upon the stimulus conditions rather than on the age of the preparations. Rhythmic activity resulting from a deteriorating preparation would not be expected to become arrhythmic again in response to altered stimulus conditions. In my experience, intracellular responses from aging preparations gradually lost their oscillatory appearance, but this was not usually seen except during extended recording sessions of 8 hours or more.

In several invertebrate preparations fluctuations in the dark potential of photoreceptors are normal components of the photoreceptor response, and their absence is considered to be a sign of a deteriorating preparation. For example, the post-illumination hyperpolarization is a normal component of the photoreceptor response in barnacle (Koike et al., 1971; Hudspeth et al., 1977; Hudspeth and Stuart, 1977) and *Limulus* (Brown and Lisman, 1972; Easland and Wasserman, 1979), and its absence has been suggested to signal deterioration of the preparation or cell damage (Easland and Wasserman, 1979). Similarly, *Limulus* photoreceptors normally show large fluctuations in dark potential in intact breathing animals, but these are not seen following excision of the eye or cutting off the blood supply (Barlow and Kaplan, 1977). The large oscillations in *Limulus* receptors are thought to represent regenerative events intrinsic to the receptor membrane (Barlow and Kaplan, 1977), while the oscillations in dragonfly ocellar receptors are thought to depend upon synaptic transmission. However, even if regenerative properties of the membrane or other non-synaptic or metabolic mechanisms contribute to the generation of spontaneous oscillations in dragonfly receptors, it seems equally probable that such oscillations would be eliminated, rather than enhanced, in damaged cells. The procedure used to sever the ocellar nerve avoided contact with more distal regions of the ocellus and did not prolong the dissection time or the time required to impale cells. Furthermore, in cut nerve preparations, stable intracellular recordings could be maintained for several hours with no apparent loss of resting potential and no deterioration of response waveform. Therefore, although it is possible

that cutting the ocellar nerve might have contributed to dark oscillatory behavior by altering a metabolic process or the ionic environment (Alkon and Grossman, 1978; Baylor and Nicholls, 1969; Erulkar and Weight, 1977; Russell and Hartline, 1978; Pinsker and Kandel, 1969; Chan et al., 1978; Baker, 1972; Bowery et al., 1979; Coles and Tsacopoulos, 1979; Kuba, 1980) of ocellar neurons, it is difficult to imagine how receptor terminals would be directly or preferentially affected by such a procedure. It seems more likely that oscillatory activity in severed L-neurons would influence photoreceptors and not vice versa.

Extracellular recordings from the lateral ocellar nerve in the dragonfly showed that spiking activity during illumination, but not OFF spikes, was abolished after severing the ocellar nerve, suggesting the former are efferent in origin (Kondo, 1978). Consequently, it could be argued that the bistable condition observed in cut nerve preparations is due to the interruption of action potentials originating in the brain. However, the elimination of postulated efferent impulses could not be the sole cause of bistable behavior because tetrodotoxin would be expected to have the same effect as cutting the ocellar nerve if spontaneous dark oscillations were dependent upon action potentials. Photoreceptors and L-neurons from tetrodotoxin treated preparations did not display any bistable behavior (Chappell, 1970; Chappell and Dowling, 1972), even though tetrodotoxin eliminated extracellular impulse activity (Jeff Rind, personal communication).

In intact nerve preparations enhanced oscillatory behavior was

only rarely seen after prolonged recording in Ringer but was commonly observed in response to the application of drugs. The most characteristic feature of such behavior in photoreceptors was the fluctuation of the interflash potential between two distinct dark equilibrium levels. Initially, the dark potential in stable photoreceptors was at a more positive level, as judged by the amplitude of the light-evoked depolarization, but gradually became more negative as the bistable condition developed. In terms of the revised feedback model presented earlier, the two levels of dark potential might correspond to two levels of feedback. The more positive level of dark potential characteristic of stable receptors could represent a maximal feedback condition, while the more negative dark potential could represent the absence of feedback.

Although two distinct "resting" potentials in one cell is not a widely reported phenomenon, a similar situation has been observed in other excitable cells. For example, two stable levels of resting potential can be induced in cardiac Purkinje fibers by drug or ionic treatments or by depolarizing shocks following such treatments (Gadsby and Cranefield, 1977). More well known examples of neurons that exhibit fluctuations in resting potentials include many invertebrate central neurons that display autoactive rhythmic activity (Pearson et al., 1975; Mendelson, 1971; Kandel, 1976; Eckert and Lux, 1976; Thompson, 1977; Ráper, 1979; Lux and Heyer, 1979). Even though such autoactive oscillatory activity is generally attributed to non-synaptic mechanisms intrinsic to the membranes of these cells, this

spontaneous activity is often modified by synaptic transmission (Pinsker and Kandel, 1969; Gillette et al., 1978; Wilson and Wachtel, 1978; Russell and Hartline, 1978).

In summary, while the ionic mechanisms responsible for the oscillatory behavior in cut nerve preparations and intact nerve preparations exposed to drugs are not known, such behavior is thought to represent an abnormal condition reflecting disruption of the dark equilibrium of a feedback loop.

In other visual systems dark oscillations in photoreceptors can be induced by a variety of experimental conditions. Barnacle photoreceptors were reported to oscillate in the presence of barium and strontium and during washout after cobalt perfusion (Ross and Stuart, 1978; Edgington and Stuart, 1979). One of the explanations offered to account for these oscillations is that strontium and barium might be inducing the release of neurotransmitter from ganglion cells (second or third order neurons) onto receptor terminals (Edgington and Stuart, 1979). In the carp retina, where a GABA mediated feedback loop from horizontal cells onto cones has been postulated (Murakami et al., 1979), cone-connected horizontal cells developed continuous dark oscillations during the application of GABA and picrotoxin (Negishi and Drujan, 1979). In the presence of picrotoxin, the same amount of GABA caused the picrotoxin-induced oscillations to cease (Negishi and Drujan, 1979). Calcium-dependent oscillations and "feedback spikes" have also been described in cones and horizontal cells of the turtle retina where they were reported to result from

synaptic feedback from horizontal cells onto cones (Piccolino and Gerschenfeld, 1977, 1980; Gerschenfeld and Piccolino, 1979, 1980). In the marine toad dark oscillations in rods and horizontal cells were suggested to result from feedback from horizontal cells onto rods (Norman and Pochobradsky, 1976). As in the dragonfly ocellus, they could be induced by light when absent and inhibited by light when present. Tetraethylammonium-induced oscillations in toad rods might also result from a voltage-dependent calcium conductance (Fain et al., 1977, 1980; Fain and Quandt, 1980).

Spontaneous dark oscillations have also been observed in L-neurons in the locust median ocellus (Wilson, 1978a,c), where they were identical in appearance to the second order oscillations reported here. In the locust, depolarizing current injected into one L-neuron evoked, with a delay, hyperpolarizing oscillations in a different, otherwise quiet cell. In addition, the same type of oscillatory behavior could also be evoked by short flashes of light. Wilson (1978c) suggested that depolarizations in the locust second order cells facilitate, with a delay, synaptic transmission from photoreceptors to L-neurons.

Post-Illumination Hyperpolarization (PIH)

A large sustained hyperpolarization following bright test flashes has not been previously described in dragonfly ocellar receptors but is normally observed in photoreceptor responses from *Limulus* (Smith et al., 1968; Brown and Lisman, 1972; Easland and Wasserman, 1979) and barnacle (Koike et al., 1970, 1971; Hudspeth and Stuart, 1977; Hudspeth et al., 1977), where it is thought to be due to an electro-

genic sodium pump (Smith et al., 1968; Koike et al., 1971; Brown and Lisman, 1972) and a long-lasting increase in potassium conductance (Hudspeth and Stuart, 1977; Hudspeth et al., 1977). The absence of a PIH in Limulus photoreceptors is thought to indicate deterioration of the preparation or cell damage (Easland and Wasserman, 1979).

It is not known whether the mechanisms responsible for generating the PIH in Limulus and barnacle are the same as those in the dragonfly, or why a PIH was always seen in cut nerve preparations but rarely seen in animals with intact nerves before the application of drugs. The sodium-potassium ATPase in many systems is known to be dependent on metabolic activity and is activated by an increase in intracellular sodium concentration or extracellular potassium concentration (Skou, 1965; Koike et al., 1971; Thomas, 1972; Brown and Lisman, 1972). Consequently, it is possible that the dragonfly PIH might reflect the activity of an electrogenic sodium pump caused by changes in the ionic environment surrounding photoreceptors, perhaps as a result of enhanced oscillatory activity in L-neurons or an increase in membrane resistance in the photoreceptor (Gorman and Mirolli, 1972; Carpenter, 1973). On the other hand, the effect of ouabain is inconsistent with such an interpretation because a PIH and bistable behavior developed in several preparations after prolonged treatment with this inhibitor of the Na^+-K^+ ATPase (Skou, 1965). At the present time, the role of an electrogenic pump in the development of the PIH in the dragonfly is inconclusive, especially since ouabain was not applied to preparations that already developed a PIH.

In bistable cells it is uncertain whether the large slow hyperpolarizations following moderate intensity stimuli (figures 39, 57, 68, 76, 79, 80) and the PIH following unattenuated stimuli (figures 35, 69, 70, 77, 81, 82) represent the same or different phenomena. In intact or cut nerve preparations the appearance of bistable behavior was usually correlated with the appearance of a PIH, but in some experiments, a PIH persisted when spontaneous dark oscillatory activity was not very pronounced or was abolished by drugs. It seems unlikely that the PIH could be solely responsible for bistable behavior because bistable cells often showed more negative dark adapted potentials after they had recovered from a PIH.

Although the mechanism responsible for generating the PIH in the dragonfly is unknown, it does not appear to be the same as that responsible for generating the normal receptor OFF response. The normal OFF-transient is usually only a few millivolts in amplitude and less than 200 msec in duration, while the PIH may be up to 20 millivolts in amplitude and last more than 30 seconds. In addition, the PIH usually appears after a delay of 800 milliseconds or more, while the OFF response is seen immediately following light-OFF. Furthermore, an OFF response was often seen in addition to a delayed sustained hyperpolarization (figures 39, 57, 68, 76, 80).

It is possible that a PIH was not normally observed in intact nerve preparations because the action of a depolarizing feedback transmitter released from L-neurons at light-OFF tended to maintain the dark potential in the photoreceptor at a more positive level

until equilibrium could be re-established. Consequently, perturbing the dark equilibrium of the feedback system by cutting the ocellar nerve or by pharmacological manipulation might induce the appearance of a PIH. The observation that during the wash period, the PIH seen after exposure to picrotoxin (figure 69) and eserine (not shown) was sometimes reversible tends to support this notion.

VII. SPECULATIONS CONCERNING A FACILITATORY FEEDBACK MECHANISM

The evidence presented in this report is consistent with a sign-conserving feedback model in which GABA facilitates the release of acetylcholine from receptor terminals. Although GABA is generally thought of as an inhibitory transmitter (Curtis and Watkins, 1965; Gerschenfeld, 1973; Krnjevic, 1974, 1976; Takeuchi, 1976; Johnston, 1978), excitatory effects of GABA have been reported in invertebrates (Kerkut and Walker, 1961, 1962; Gerschenfeld and Lasansky, 1964; Walker et al., 1975; Yarowsky and Carpenter, 1977), and an excitatory role for GABA has also been suggested in vertebrates (Obata, 1976). In addition, GABA mediated depolarizations are commonly found within the vertebrate central nervous system (DeGroat, 1970, 1972; Barker and Nicoll, 1972; Adams and Brown, 1975; Obata, 1976; Simmonds, 1978; Pickles, 1978; Levy, 1974, 1979; Alger and Nicoll, 1979), and a depolarizing action of GABA on second order neurons in the fly compound eye has been proposed (Zimmerman, 1978).

Recent evidence indicates that very small depolarizations (1-2 mv or less) are effective in releasing neurotransmitter from photoreceptors (Jarvilehto and Zettler, 1971; Shaw, 1972; Laughlin, 1973; Ashmore and Falk, 1976; Ross and Stuart, 1978; Stuart and Oertel, 1978; Baylor and Fettiplace, 1977) and other neurons (Bennett, 1968; Bush and Roberts, 1968; Ripley et al., 1968; Pearson and Fourtner, 1975; Burrows and Siegler, 1976; Schmitt et al., 1976; Burrows, 1979; Graubard and Calvin, 1979) in which synaptic transmission is mediated by slow potentials. Therefore, GABA could facilitate the release of

receptor transmitter by direct depolarization of the receptor terminal, as the antidromic stimulation experiments and pharmacological evidence suggest.

A feedback transmitter that acted to decrease the conductance of the receptor terminal (Carew and Kandel, 1976; Schulman and Weight, 1976) could also have a facilitatory effect by augmenting small depolarizations induced by light or other mechanisms. A depolarizing GABA mediated response associated with a conductance decrease has been described in *Aplysia* (Yarowsky and Carpenter, 1977) and *Onchidium* (Oomura et al., 1974). Acetylcholine (Weight and Votova, 1970; Krnjevic et al., 1971; Schulman and Weight, 1976) and serotonin (Gerschenfeld and Paupardin-Tritsch, 1974a) have also been reported to mediate depolarizing responses by decreasing the conductance of the postsynaptic membrane.

In recent years several neurotransmitters have been shown to act not simply by exciting or inhibiting an electrically excitable cell but by modulating the effects of other events occurring at the cell (Greengard, 1978; Kupferman, 1979). In *Aplysia* neurons serotonin mediates presynaptic facilitation by stimulating the formation of cyclic AMP inside the presynaptic terminal (Castellucci and Kandel, 1976; Brunelli et al., 1976), presumably by facilitating calcium entry or otherwise by increasing the concentration of intracellular calcium at the presynaptic terminal. Serotonin has also been reported to excite by inducing voltage sensitive ion channels to sodium (Pellmar and Wilson, 1977) or facilitating a voltage sensitive calcium current (Kandel, 1976; Brunelli et al., 1976; Alkon, 1979). In *Hermissenda*

a persistent depolarization and increased membrane resistance in type B photoreceptors is thought to be dependent on synaptic and metabolic mechanisms and is also sensitive to intracellular cyclic AMP (Alkon, 1979). It is also conceivable that a facilitatory neurotransmitter could act by increasing the synthesis of a transmitter or by reducing its uptake or inactivation.

In summary, there are several mechanisms whereby GABA might facilitate the release of acetylcholine from receptor terminals in the dragonfly ocellus. Conclusive identification of ocellar neurotransmitters, as well as their mechanisms of action, will require additional experiments utilizing dual recording, biochemical, and histochemical techniques.

VIII. CONCLUSION

The evidence presented in this report strongly suggests there is synaptic feedback onto photoreceptors in the median ocellus of the dragonfly. This evidence includes: 1) Hyperpolarizing the ocellar nerve with extrinsic current induces a typical hyperpolarizing OFF oscillation in the dark adapted intracellular photoreceptor response. 2) The normal receptor response is modified by pharmacological agents shown to modify the response of the L-neuron. 3) Dark oscillations in L-neurons are correlated with similar dark oscillations in photoreceptors and are also modified by the same pharmacological agents. 4) A bistable condition induced by pharmacological manipulation or cutting the ocellar nerve is susceptible to modification by drugs. The electrophysiological and pharmacological data are consistent with the hypothesis that the receptor transmitter may be acetylcholine and the feedback transmitter might be GABA. These findings support a sign-conserving feedback model in which GABA facilitates the release of acetylcholine from the receptor terminal when the L-neuron is depolarized in the dark. However, not all of the experimental findings are consistent with a simple feedback model but can be explained by considering lateral synaptic interactions between receptors and L-neurons. The prolonged conductance increase associated with the depolarizing L-neuron OFF response suggests that the L-neuron transmitter (GABA) also depolarizes adjacent L-neuron dendrites. During exposure to cholinergic agonists (as during background illumination), the reduction in the L-neuron light response, and the absence of

sustained hyperpolarization, leads to the suggestion that the receptor transmitter (acetylcholine) may have an inhibitory effect on adjacent receptor terminals. A revised feedback model, including both feedback and lateral interactions (see figure 83), is supported by anatomical studies describing numerous lateral as well as feedback synapses between L-neurons and receptor terminals (Dowling and Chappell, 1972).

Much of the evidence in the dragonfly suggests that facilitatory feedback onto photoreceptors and depolarizing lateral interactions between L-neurons are more important in the dark (or under conditions in which there is a reduction in illumination), while feed-forward inhibitory interactions from photoreceptors to L-neurons and lateral inhibitory interactions between adjacent receptor terminals are more important during strong illumination (or under conditions in which there is an increase in illumination). Such a system of feedback could play a critical role in enhancing the response to transient changes in illumination over a widely varying range in constant background conditions.

A central, and as yet, unresolved problem in retinal neurophysiology is the manner in which sustained responses are transformed into phasic responses as visual information passes from one cell type to another on its way to the brain. The feedback loop in the dragonfly could contribute to transforming the sustained depolarizing response in the photoreceptor into the more phasic, hyperpolarizing response of the L-neuron. The dark oscillatory behavior in ocellar retinal neurons may be a consequence of disrupting the dark equilibrium of a feedback loop, either by pharmacological manipulation or by

isolating the L-neuron processes from the brain.

Oscillatory potentials in invertebrate (Lang and Hartline, 1974; Barlow and Fraioli, 1978; Wilson, 1978b,c) and vertebrate (Adrian and Matthews, 1928; Brindley, 1956; Graham and Pong, 1972; Ogden, 1973; Norman and Pochobradsky, 1976; Wachmeister and Dowling, 1978; Gerschenfeld and Piccolino, 1980) retinal recordings have been of interest for some time and are also thought to be generated by lateral and/or feedback synaptic interactions within the retina. In addition, evidence from several laboratories indicates that synaptic feedback from horizontal cells onto cones may play an important role in the processing of visual information in some vertebrate retinas (Baylor et al., 1971; Fuortes et al., 1973; Simon, 1974; Pinto and Pak, 1974; Kleinschmidt and Dowling, 1975; Burkhardt, 1977; Thibos and Werblin, 1978; Hedden and Dowling, 1978; Piccolino and Gerschenfeld, 1977, 1980; Gerschenfeld and Piccolino, 1979, 1980), and in several species the feedback transmitter released from horizontal cells onto cones has also been suggested to be GABA (Murakami et al., 1978; Marc et al., 1978; Lam et al., 1979a,b; Belgum and McReynolds, 1979; Wu and Dowling, 1979).

In vertebrates, feedback from horizontal cells onto cones is thought to inhibit the release of cone transmitter (Murakami et al., 1978; Gerschenfeld and Piccolino, 1980) rather than to facilitate the release of receptor transmitter as in the dragonfly. The sign of the photoreceptor light response (dragonfly receptors depolarize; cones hyperpolarize) and receptor transmitter action (dragonfly receptor transmitter inhibits L-neurons; cone transmitter excites horizontal

cells) is opposite in the dragonfly ocellus and the vertebrate retina. As a consequence, however, certain similarities result. Since the second order neurons are depolarized in the dark in both cases, both can be expected to release maximum amounts of feedback transmitter in the dark. In each case, cell dark potentials may represent an equilibrium potential established by this feedback loop. Light will reduce the release of feedback transmitter in each case, which will tend to extend the dynamic range of the system while enhancing responses to transient changes in illumination.

My experimental results, in conjunction with earlier anatomical (Dowling and Chappell, 1972; Patterson and Chappell, 1980), electrophysiological (Chappell and Dowling, 1972), and pharmacological (Klingman and Chappell, 1978) studies provide fairly conclusive evidence that the intracellular OFF responses recorded from ocellar retinal neurons are generated by graded synaptic interactions originating in the ocellar plexiform layer, as first suggested by Chappell and Dowling (Chappell, 1970; Chappell and Dowling, 1972; Dowling and Chappell, 1972).

In recent years signal transmission by graded electrotonic conduction and local circuit interactions have been suggested to play an important role in information processing in many regions of the nervous system (Schmitt et al., 1976; Shepherd, 1979). The median ocellus of the dragonfly is an ideal model system for learning more about the properties of local circuit interactions, which are currently of great interest in neurobiology.

Topical areas of investigation for which the dragonfly median ocellus is especially well suited include:

1. function and properties of spikeless synaptic transmission.
2. role of non-spiking neurons.
3. mechanisms involved in slow potential mediated release of neurotransmitter.
4. presynaptic function of dendrites.
5. dendro-dendritic and axo-axonic interactions.
6. short- and long-term transmitter actions.

In addition, the dragonfly ocellus is one of the few preparations where the properties of dyad synapses are amenable to thorough analysis.

During the course of my experiments several observations were noted which should prove valuable in future investigations. First, the discovery that the level of the photoreceptor light response is remarkably constant can be used as a technique for determining changes in the receptor resting potential during prolonged recording sessions. The finding that light acts as a "current clamp" on the intracellular receptor response can help eliminate uncertainties due to random shifts in the baseline level of oscilloscope trace, which is a common problem in electrophysiological studies. Consequently, changes in the level of the receptor dark potential can be measured by superimposing the light responses and using the sustained level of the light response as a reference point for comparing changes in the receptor dark potential.

A second useful byproduct of my pharmacological experiments was the observation that in cut nerve preparations, the time course of a drug study was more rapid and required lower drug concentrations than in intact nerve preparations. This is probably because penetration of a drug is facilitated when the sheath surrounding the ocellus and brain is interrupted. These findings suggest that the cut ocellar nerve preparation may prove to be a useful technique in future pharmacological studies since the probability that an experiment can be completed (before a cell is lost) is greatly increased if drug effects are detected earlier and are more readily reversible.

In conclusion, my results provide strong evidence for synaptic feedback onto photoreceptors in the ocellar retina of the dragonfly. My data is not presumed to be sufficient to conclusively identify the neurotransmitters involved in the feedback loop or their mechanism of action. Nevertheless, at the present time the revised feedback model described earlier in this report seems adequate to account for the electrophysiological and pharmacological findings and is consistent with the available literature. One of the major advantages of such a model is to provide a framework for future investigations in which old and new ideas relating to feedback interactions can be tested. The basic mechanisms responsible for my results should prove to be important, even if it turns out they can be better explained by alternative means. The median ocellus of the dragonfly is an excellent model system for learning more of graded synaptic transmission and local circuit interactions in the retina and elsewhere in the brain.

NOTES

1. The ocellar nerve of the dragonfly (Cajal, 1918; Ruck and Edwards, 1964; Kirkham et al., 1975), locust (C.S. Goodman, 1974, 1976a,b; C.S. Goodman and Williams, 1976) and honey bee (Pan and Goodman, 1977) is comprised of several large second order fibers and more numerous (25-40) smaller ocellar nerve fibers which are the processes of two populations of large and small second order neurons respectively (C.S. Goodman, 1976a; C.S. Goodman and Williams, 1976; Guy et al., 1979). In the ocellus of the dragonfly (Dowling and Chappell, 1972) and locust (L.J. Goodman, 1979) the large second order neurons make feedback synapses onto receptor terminals, but in the worker honey bee (Guy et al., 1979) the smaller second order processes appear to make feedback synapses onto receptor terminals. It has been suggested that the smaller ocellar nerve processes constitute a separate class of ocellar neuron that may be the source of the intracellular and extracellular spiking activity (Rosser, 1974; Wilson, 1978a; Kondo, 1978; Guy et al., 1979). At the present time spiking processes have not been identified by intracellular recording and dye injection. Consequently, the question as to whether they constitute a functionally distinct class of ocellar retinal neuron has not been resolved. Furthermore, if they do represent a physiologically distinct class of neuron, there is no reason to assume that they serve an identical function in the ocelli of all species (Wilson,

1978a; Guy et al., 1979). In the dragonfly, impulse activity does not appear to significantly influence information processing in the distal plexiform layer because L-neuron dendrite responses from cut nerve preparations show similar pharmacological and electrophysiological behavior to those recorded from intact nerve preparations, and L-neuron responses were not obviously modified by tetrodotoxin (Chappell and Dowling, 1972) which also blocks extracellular impulse activity (Jeff Rind, personal communication).

2. In the barnacle, the second order responses show hyperpolarizing waveform associated with a conductance increase during illumination and tetrodotoxin insensitive impulses at light OFF ("I cell") while third order neurons ("A cell") show hyperpolarizing responses to light (associated with a conductance decrease) and tetrodotoxin sensitive impulses at light OFF (Stuart and Oertel, 1978; Oertel and Stuart, 1981).

3. Intracellular responses showing light-excited and light-inhibited impulse activity have been recorded from the median ocellus of the locust (Wilson, 1978a) and worker honey bee (Guy et al., 1979), where they were suggested to correspond to the extracellular impulse activity that is readily recorded from the median (Chappell and Dowling, 1972) and lateral (Ruck, 1961a,b,c; Rosser, 1974; Kondo, 1978) ocellus in the dragonfly. It was further suggested that the source of the intracellularly recorded impulses was the small ocellar nerve fibers, which might constitute a functionally

as well as anatomically distinct class of cell (Wilson, 1978a; Kondo, 1978; Guy et al., 1979). However, none of these spiking cells have been identified by intracellular dye injection, and penetration of these processes was rare and could be maintained for only seconds or at most minutes (Wilson, 1978a). "Consequently, nothing beyond their existence has been established" (Wilson, 1978a). In addition, none of the smaller ocellar nerve fibers have been both anatomically and electrophysiologically identified by intracellular dye injection. Therefore, the source of such spiking units must remain speculative until more evidence is available.

Several alternative explanations might account for the rarely recorded spiking processes in the dragonfly ocellus:

(1) The impulses recorded on rare occasions may be generated at a distant site in the brain. Action potentials originating in a different cell may be conducted electronically along the membrane of the L-neuron where they are picked up by the recording electrode. A similar explanation was one of those suggested by Wilson (1978b) to account for the spike-like "fast OFF transient" in the locust L-neuron response. This type of situation is not uncommon. For example, miniature spikes are commonly observed in intracellular recordings from the retinula cells of the lateral eye of <u>Limulus</u> (Easland and Wasserman, 1979). They represent the electrotonic spread of spike currents in the eccentric cell across

gap junctions into the retinula cell (Smith and Bauman, 1969), and the ability to detect them appears to be a function of the site of the recording electrode (Barlow and Kaplan, 1977).

(2) Ocellar neurons, which are normally non-spiking, may be capable of generating action potentials under unusual circumstances. Hengstenberg (1978) has found that light-induced depolarizations in lobula plate interneurons are normally not associated with spiking activity, but will generate spikes during illumination when the membrane of the cell is hyperpolarized by current injection. In the vertebrate (Fain et al., 1977, 1980; Fain and Quandt, 1980; Piccolino and Gerschenfeld, 1980; Gerschenfeld and Piccolino, 1980) and invertebrate (Ross and Stuart, 1978; Edgington and Stuart, 1979; Cornwall and Gorman, 1979) retina, calcium spikes (Hagiwara, 1973) in non-spiking neurons can be elicited under non-physiological conditions.

(3) L-neuron dendrites within the ocellar plexiform layer may not normally generate action potentials, while more proximal regions of the L-neuron may be capable of impulse activity.

It has recently been suggested that different regions of the same neuron may function independently in the processing of visual information (Nelson et al., 1975; Pearson, 1979; Weiler and Zettler, 1979). There-

fore, it is conceivable that more proximal regions of the L-neuron may generate spikes which might be detected by the recording electrode on rare occasions. However, Mobbs et al. (1981) have found that L-neuron responses recorded from the dragonfly brain also do not show impulse activity.

(4) Intracellular spiking activity may be the result of occasional damage to the cell. For example, the cells showing impulse activity during illumination might be originating in small photoreceptor terminals that were damaged by the microelectrode. The light-stimulated spikes (figure 20) were impaled in the same region of the ocellus where presumed receptor terminals were impaled, and receptors are known to be capable of generating spikes because an ON-spike is commonly observed in the dragonfly ocellar receptor response (Chappell and Dowling, 1972). Similarly, light-inhibited spiking units may be originating in damaged L-neurons.

In summary, the situation regarding the origin and nature of the intracellular impulses recorded from the synaptic region of the ocellus is confusing. It is not known if the intracellularly recorded spikes (figures 20, 21) correspond to extracellular impulse activity that is readily recorded from the median (Chappell and Dowling, 1972) and lateral ocelli (Ruck, 1961a,b,c; Rosser, 1974; Kondo, 1978) and is sensitive to tetrodotoxin (Jeff Rind,

personal communication) or if they represent a different type of regenerative activity (Stuart and Oertel, 1978) of uncertain function (Wilson, 1978b). Furthermore, since no spiking unit in an insect ocellus has been stained, it has not been determined if action potentials are a normal component of some distant region of the L-neuron, or a normal component of a third class of ocellar neuron. It is also possible that the intracellular impulses are an abnormal component of a neuron that is normally non-spiking (Hengstenberg, 1978) or the result of occasional damage to a cell. These alternatives cannot be distinguished without additional experimental evidence.

4. Lisman and Brown (1971) describe a "slow process" in *Limulus* ventral photoreceptors in which light induces a decrease in a voltage-dependent potassium conductance (Leonard and Lisman, 1981). A light induced decrease in a voltage-dependent potassium conductance might explain why the receptor OFF response in the dragonfly ocellus is normally more prominent following intermediate light intensities but cannot explain the observed enhancement of the OFF hyperpolarization in response to increasing the duration and frequency of the test flash. On the contrary, such a mechanism should *reduce* an OFF hyperpolarization due to a voltage-dependent potassium conductance which is decreased by light. Furthermore, in *Limulus* the time course of the "slow process" occurred slowly

(seconds) and recovered in minutes (Lisman and Brown, 1971; Leonard and Lisman, 1981) which argues against a similar process being responsible for the receptor OFF response in the dragonfly.

Baumann and Hadjilazaro (1972) also describe both a hyperpolarizing and depolarizing after effect of intense light in the receptor of the honeybee drone compound eye. They suggest that the depolarizing after potential is caused by the same process as the plateau of the receptor potential.

5. Wilson (1978b) noted that L-neuron OFF response in the locust median ocellus often has a spike-like appearance, which was especially distinct when recording L-neuron responses from the ocellar nerve. He suggested that the L-neuron OFF response represents three separate events:

   (1) The "fast OFF transient" was the earliest component of the OFF response and had a spike-like appearance. It was suggested that "fast OFF transients" are due to "action potentials generated at a site in the brain and conducted passively, with considerable attenuation along the ocellar nerve fibers to the site of the recording electrode."

   (2) The "slow OFF transient" referred to the slow depolarizing phase of the OFF response and was presumed to be mediated by synaptic transmission. The "slow OFF transient" was associated with a conductance increase and could not be reversed by hyperpolarizing or depolarizing the membrane of the second order cell.

(3) An additional phase of the second order OFF response "preceding the slow OFF transient" was reversed by hyperpolarizing the membrane of the second order neuron to potentials more negative than the reversal potential for the light ON portion of the response. This early component of the L-neuron OFF response was suggested to correspond to the period immediately following light OFF when no receptor transmitter was being released.

6. Multiple actions of a single neurotransmitter. A neuron can have more than one type of receptor to the same neurotransmitter. The action of a neurotransmitter depends upon the nature of the receptive site (and/or ionophore) on the post-synaptic membrane. Thus a single transmitter can produce opposite effects and several different transmitters are capable of mediating the same post-synaptic response (see Kandel, 1976). It is now known that a single neurotransmitter can act to hyperpolarize or depolarize a post-synaptic cell by increasing or decreasing conductance or by inducing a metabolic or biochemical event that is not associated with a change in conductance (Chiarandini and Gerschenfeld, 1967; Gerschenfeld, 1973; Krnjevic, 1974; Kandel, 1976; Brunelli et al., 1976; Schulman and Weight, 1976; Pellman and Wilson, 1977; Marshall and Engberg, 1979; Kupferman, 1979).

In vertebrates, acetylcholine receptors are classified as nicotinic or muscarinic, based on the ability of the alkaloids nicotine or muscarine to bind to the acetylcholine receptor on

the post-synaptic membrane (Dale, 1914; Koelle, 1975a,b,c,d; Volle and Koelle, 1975). In vertebrates, nicotinic type responses to acetylcholine are generally excitatory (Krnjevic, 1974; Koelle, 1975a,d) but in invertebrates nicotinic-like acetylcholine responses can be excitatory or inhibitory (Tauc and Gerschenfeld, 1961, 1962; Kehoe, 1972a,b,c; Levitan and Tauc, 1972; Gardner and Kandel, 1972) and are often blocked by atropine (a muscarinic acetylcholine antagonist) as well as curare (Tauc and Gerschenfeld, 1961, 1962; Pinsker and Kandel, 1967; Kehoe, 1972a,b,c). Furthermore, in some invertebrate neurons, different nicotinic type acetylcholine receptors may show differential sensitivity to curare (Gardner and Kandel, 1972; Kehoe, 1972b; Levitan and Tauc, 1972). In both invertebrates (Tauc and Gerschenfeld, 1961, 1962; Kehoe, 1972a,b,c; Gerschenfeld, 1973; Kandel, 1976) and vertebrates (Curtis and Ryall, 1966; Eccles and Libit, 1961; Weight and Votova, 1970; Schulman and Weight, 1976) more than one class of acetylcholine receptor may be located on the same cell.

More than one class of receptive site has also been suggested for GABA (Walker et al., 1975; Azanza and Walker, 1975; Yarowsky and Carpenter, 1977; Alger and Nicoll, 1979; Bowery et al., 1980; Marder and Paupardin-Tritsch, 1980), serotonin (Gerschenfeld and Paupardin-Tritsch, 1974a,b; Brunelli et al., 1976), dopamine (Ascher, 1972; Ascher and Kehoe, 1975; Berry and Cotrell, 1975), and nor-epinepherine (Marshall and Engberg, 1978).

Biphasic drug effects during pharmacological studies can sometimes be attributed to multiple actions of a neurotransmitter on

the same cell (Ascher, 1972; Gardner and Kandel, 1972; Kehoe, 1972b,c; Berry and Cottrell, 1975; Azanza and Walker, 1975; Kandel, 1976; Yarowsky and Carpenter, 1977; Alger and Nicoll, 1979).

7. Additional actions of curare. Curare has been reported to block the effects of several neurotransmitters in addition to acetylcholine. For example, curare was reported to block the action of serotonin (Gerschenfeld and Paupardin-Tritsch, 1974a,b; Ascher and Kehoe, 1975; Carpenter et al., 1977), dopamine (Ascher, 1972; Ascher and Kehoe, 1975; Carpenter et al., 1977), GABA (Carpenter et al., 1977; Yarowsky and Carpenter, 1977) and as many as nine putative neurotransmitters (Carpenter et al., 1977). On the other hand, some cholinergic responses are not blocked by curare in *Aplysia* neurones although they are mimicked by carbachol (Kehoe, 1972b).

8. Additional actions of picrotoxin. Picrotoxin has been reported to block the action of several neurotransmitters (other than GABA) including acetylcholine (Yarowsky and Carpenter, 1978; Marder and Paupardin-Tritsch, 1980), serotonin (deGroat and Lalley, 1973; deGroat and Simmonds, 1976), glutamate (Florey and Murdock, 1974; Marder and Paupardin-Tritsch, 1978), glycine (Hill et al., 1976) and alanine and taurine (Barker et al., 1975).

Furthermore, picrotoxin may have additional non-specific effects or side effects. Not all GABA mediated responses are

blocked by picrotoxin (Yarowsky and Carpenter, 1977; Marder and Paupardin-Tritsch, 1978; Constanti, 1978; Alger and Nicoll, 1979) and picrotoxin may have direct actions on neuronal membranes (Grundfest et al., 1959; Takeuchi and Takeuchi, 1969; Krnjevic et al., 1966; Olsen et al., 1975, 1978; Tiku and Olsen, 1977; Fremman, 1973; Marder and Paupardin-Tritsch, 1979; Barker and MacDonald, 1980) or may interfere with non-synaptic GABAergic binding sites on pre-synaptic terminals (Simmonds, 1978; Pickles, 1979). Picrotoxin has also been reported to elevate the level of acetylcholinesterase in mammalian brain (Svenneby and Roberts, 1973, 1974). However, non-specific actions of picrotoxin and other GABA antagonists differ in different species, and at different sites in the same species (Gerschenfeld, 1973; Krnjevic, 1974; Levy, 1977).

9. Desensitization. Desensitization is the general term used by physiologists and pharmacologists for the progressive loss of responsiveness during the sustained application of neurotransmitters or agonists.

Desensitization of acetylcholine receptors is well known in vertebrates (Katz and Thesleff, 1957; Krnjevic, 1974; Feltz and Trautman, 1980) and invertebrates (Gardner and Kandel, 1972; Callec and Sattelle, 1973; Sattelle et al., 1976; Yarowsky and Carpenter, 1978; Bregestovski et al., 1979). Therefore the possibility that the reduced L-neuron response during the application of cholinergic agents was the result of desensitization cannot be eliminated.

Desensitization of GABAergic receptors has also been reported in invertebrates (Epstein and Grundfest, 1970; Gerschenfeld, 1973; Walker et al., 1975; Azanza and Walker, 1975; Yarowsky and Carpenter, 1978) and vertebrates (Krnjevic, 1974; Adams and Brown, 1975; Levy, 1977; Murakami et al., 1978).

10. Agonistic-antagonistic effects. Many drugs that bind to a receptor can act as agonists or antagonists, and some can produce two effects in sequence. Nicotine is a well known example of such an agent (Koelle, 1975a; Volle and Koelle, 1975). At vertebrate autonomic ganglia low doses of nicotine have stimulating effects while high doses result in a blocking action. A blocking action of nicotine has also been reported for hyperpolarizing acetylcholine receptors in *Aplysia* neurons (Kehoe, 1972b). Both eserine and carbachol have also been reported to have blocking actions at pre-synaptic and post-synaptic sites. The importance of these direct actions varies with the drug, dose, species and site (Patton and Perry, 1953; Koelle, 1975a,b,c,d; Volle and Koelle, 1975; Gerschenfeld, 1979).

11. Non-junctional (non-synaptic) binding sites for neurotransmitters and drugs. Non-junctional binding sites for neurotransmitters and related drugs have been reported on presynaptic terminals and elsewhere on a neuron (Koelle, 1961, 1962; Blaber, 1972; Miyamoto and Volle, 1974; Miyamoto, Adams and Brown, 1975; Miyamoto, 1978; Simmonds, 1978; Pickles, 1979; Glavinovic, 1979; Duncan and

Publicover, 1979). In most cases it is not known if extra-junctional receptors for neurotransmitters and related drugs are of physiological significance. However, the presence of such non-synaptic binding sites for drugs can sometimes account for anomalous drug effects during pharmacological studies.

12. Drug-receptor interactions. Many factors can affect the binding of neurotransmitters, agonists, and antagonists. The exact mechanisms of action are different in different species, and at different sites in the same species, and depend upon the nature of the drug, the concentration, and the presence of other drugs (Gerschenfeld, 1973; Krnjevic, 1974; Koelle, 1975a,b,c,d; Volle and Koelle, 1975; Levy, 1977).

The surface potential on the membrane containing the receptor molecule can influence the binding of transmitters, agonists and antagonists, and many curious pharmacological effects might be due to modification of the surface charges on the membrane by the ionic composition of the bathing medium and the valences of the drugs (Van der Kloot and Cohen, 1979). At some sites the presence of other drugs (Feltz and Trautman, 1980; Yarowsky and Carpenter, 1978), the nature of the ionophore (Takeuchi and Takeuchi, 1969; Freeman, 1973; Olsen et al., 1978; Yarowsky and Carpenter, 1978) and membrane potential and the nature of the permeant ion (Marty et al., 1976; Manalis, 1977; Ascher et al., 1978, 1979; Marchais et al., 1979; Colquhoun et al., 1979) might also contribute to the action of a drug.

13. The electrophysiological evidence is not consistent with the idea that shape of the L-neuron light response is caused by an opposite transmitter action acting directly on the L-neuron membrane during illumination. For example, it could be argued that the cut back from ON-transient to plateau in the L-neuron response is caused by a neurotransmitter that depolarizes during illumination by an increased conductance mechanism. However, the evidence suggests that the cut back from ON-transient to plateau is due to a decrease in the amount of inhibitory receptor transmitter released rather than a depolarization caused by an excitatory transmitter that increases conductance to an ion or ions with a positive equilibrium potential. This is because the plateau portion of the L-neuron light response in the locust always reversed at the same or more negative potentials than the reversal potential for the ON-transient portion of the light response (Wilson, 1978b). Therefore, the ionic mechanism responsible for the plateau portion of the L-neuron light response is probably the same mechanism responsible for the ON-transient portion of the light response. The receptor transmitter apparently hyperpolarizes the L-neuron membrane by increasing conductance to an ion with a negative equilibrium potential (Klingman and Chappell, 1978; Wilson, 1978b) and the plateau portion of the L-neuron light response corresponds to a period in which the quantity of this receptor transmitter is reduced (Klingman and Chappell, 1978; Wilson, 1978b).

REFERENCES

Adams, P.R. and Brown, D.A. (1975). Actions of γ-aminobutyric acid on sympathetic ganglion cells. J. Physiol. (Lond.) 250:85-120.

Adrian, E.D. and Matthews, R. (1928). The action of light on the eye. Part III. The interaction of retinal neurones. J. Physiol. (Lond.) 65:278-298.

Alger, B.E. and Nicoll, R.A. (1979). GABA-mediated biphasic inhibitory responses in hippocampus. Nature 281:315-317.

Alkon, D.L. (1974). Sensory interactions in the nudibranch mollusk Hermissenda crassicornis. Fed. Proc. 33:1083-1090.

Alkon, D.L. (1979). Voltage-dependent calcium and potassium ion conductances: A contingency mechanism for an associative learning model. Science 205:810-816.

Alkon, D.L. and Fuortes, M.G.F. (1972). Responses of photoreceptors in Hermissenda. J. gen. Physiol. 60:631-649.

Alkon, D. and Grossman, Y. (1978). Evidence for non-synaptic neural interaction. J. Neurophysiol. 41:640-653.

Armett-Kibel, C., Meinertzhagen, I.A. and Dowling, J.E. (1977). Cellular and synaptic organization in the lamina of the dragonfly Sympetrum rubicundulum. Proc. Roy. Soc. Lond. B196:385-413.

Ascher, P. (1972). Inhibitory and excitatory effects of dopamine on Aplysia neurones. J. Physiol. (Lond.) 225:173-209.

Ascher, P. and Kehoe, J.S. (1975). Amine and amino-acid receptors in gastropod neurons. In: Handbook of Psychopharmacology, vol.4, Amino acid neurotransmitters. Iversen, L.L., Iversen, S.D. and Snyder, S.H. (Eds.). New York: Plenum Press, pp. 265-316.

Ascher, P., Large, W.A. and Rang, H.P. (1979). Studies on the mechanism of action of acetylcholine antagonists on rat parasympathetic ganglion cells. J. Physiol. (Lond.) 295:139-170.

Ascher, P., Marty, A. and Neild, T.O. (1978). The mode of action of antagonists of the excitatory response to acetylcholine in Aplysia neurons. J. Physiol. (Lond.) 278:207-235.

Ashmore, J.F. and Falk, G. (1976). Absolute sensitivity of rod bipolar cells in a dark-adapted retina. Nature 263:248-249.

Autrum, H., Zettler, F. and Jarvilehto, M. (1970). Postsynaptic potentials from a single mono-polar neuron of the ganglion opticum I of the blowfly Calliphora. Z. Vergl. Physiol. 70:414-424.

Azanza, M.J. and Walker, R.J. (1975). GABA-receptor interactions; models in Helix asperas neurons. Comp. Biochem. Physiol. 50C:155-161.

Baker, P.F. (1972). Transport and metabolism of calcium ions in nerve. Prog. Biophys. Mol. Biol. 24:177-223.

Baker, P.F., Blaustein, M.P., Hodgkin, A.L. and Steinhardt, R.A. (1969). Influence of calcium on sodium efflux in squid axons. J. Physiol. (Lond.) 200:431-458.

Baker, P.F., Hodgkin, A.L. and Ridgway, E.B. (1971). Depolarization and calcium entry in squid giant axons. J. Physiol. (Lond.) 218:709-755.

Barker, J.L. and MacDonald, J.F. (1980). Picrotoxin convulsions involve synaptic and non-synaptic mechanisms on cultured mouse spinal neurons. Science 208:1054-1056.

Barker, J.L. and Nicoll, R.A. (1972). Gamma-aminobutyric acid: role in primary afferent depolarization. Science 176:1043-1045.

Barker, J.L., Nicoll, R.A. and Padjen, A. (1975). Studies on convulsants in the isolated frog spinal cord. I. Antagonism of amino acid responses. J. Physiol. (Lond.) 245:521-536.

Barlow, R.B. and Kaplan, E. (1977). Properties of visual cells in the lateral eye of Limulus in situ. J. gen. Physiol. 69:203-220.

Barlow, R.B. and Fraioli, A.J. (1978). Inhibition in the Limulus lateral eye in situ. J. gen. Physiol. 75:699-720.

Baumann, F. (1968). Slow and spike potentials recorded from retinula cells of the honey bee drone in response to light. J. gen. Physiol. 52:855-875.

Baumann, F., and Hadjilazaro, B. (1972). A depolarizing aftereffect of intense light in the drone visual receptor. Vision Res. 12:17-31.

Baylor, D.A. and Fettiplace, R. (1977). Transmission from photoreceptors to ganglion cells in the turtle retina. J. Physiol. (Lond.) 271:391-424.

Baylor, D.A. and Fuortes, M.G.F. (1970). Electrical responses of single cones in the retina of the turtle. J. Physiol. (Lond.) 207:77-92.

Baylor, D.A., Fuortes, M.G.F. and O'Bryan, P.M. (1971). Receptive fields of cones in the retina of the turtle. J. Physiol. (Lond.) 214:265-294.

Baylor, D.A. and Nicholls, J.G. (1969). Changes in extracellular potassium concentration produced by neuronal activity in the central nervous system of the leech. J. Physiol. (Lond.) 203:571-589.

Belgum, J.H. and McReynolds, J.S. (1979). Synaptic transmission in the outer plexiform layer of Necturus retina. Ass. Res. Vis. Opthalmol. (Abstr.), p. 32.

Bennett, M.V.L. (1968). Similarities between chemically and electrically mediated transmission. In: Physiological and Biochemical Aspects of Nervous Integration, Carlson, F.D. (Ed.). New Jersey: Prentice-Hall, pp. 73-128.

Berry, M.S. and Cottrell, G.A. (1975). Excitatory, inhibitory and biphasic synaptic potentials mediated by an identified dopamine containing neurone. J. Physiol. (Lond.) 244:589-612.

Blaber, L.C. (1972). The mechanism of the facilitatory action of edrophonium in cat skeletal muscle. Br. J. Pharmac. 46:498-507.

Bortoff, A. (1964). Localisation of slow potentials in the Necturus retina. Vision Res. 4:677-635.

Bowery, N.G., Brown, D.A. and Marsh, S. (1979). γ-aminobutyric acid efflux from sympathetic glial cells: Effect of "depolarizing" agents. J. Physiol. 293:75-101.

Bowery, N.G., Hill, D.R., Hudson, A.L., Doble, A., Middlemiss, D.N., Shaw, J. and Turnbull, M. (1980). (-) Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature 283:92-94.

Bregestovski, P.D., Bukharaeva, E.A. and Iljin, V.I. (1979). Voltage clamp analysis of acetylcholine receptor desensitization in isolated mollusc neurons. J. Physiol. (Lond.) 297:581-595.

Brehm, P. and Eckert, R. (1978). Calcium entry leads to inactivation of calcium channel in paramecium. Science 202:1203-1206.

Brindley, G.S. (1956). Responses to illumination recorded by microelectrodes from the frog's retina. J. Physiol. (Lond.) 134:360-384.

Brown, H.M., Hagiwara, S., Kolke, H. and Meech, R.W. (1970). Membrane properties of a barnacle photoreceptor examined by voltage-clamp technique. J. Physiol. (Lond.) 208:385-413.

Brown, J.E. and Lisman, J.E. (1972). An electrogenic sodium pump in Limulus ventral photoreceptor cells. J. gen. Physiol. 59:720-733.

Brown, J.E. and Mote, M.E. (1974). Ionic dependence of reversal voltage of light response in Limulus photoreceptors. J. gen. Physiol. 64:337-350.

Brownstein, M.J., Saavedra, J., Axelrod, J., Zeman, G.H. and Carpenter, D.O. (1974). Coexistence of several putative neurotransmitters in a single identified neuron of Aplysia. Proc. Natl. Acad. Sci. USA 71:4662-4665.

Brunelli, M., Castellucci, V. and Kandel, E.R. (1976). Synaptic facilitation and behavioral sensitization in Aplysia: Possible role of serotonin and cyclic AMP. Science 194:1178-1180.

Burkhardt, D. (1962). Spectral sensitivity and other response characteristics of single visual cells in the arthropod eye. Symp. Soc. Exp. Biol. 16:86-109.

Burkhardt, D.A. (1977). Responses and receptive-field organization of cones in perch retina. J. Neurophysiol. 40:53-62.

Burrows, M. and Siegler, M.V.S. (1976). Transmission without spikes between locust interneurones and motor neurones. Nature 262:222-224.

Burrows, M. (1979). Synaptic potentials affect the release of transmitter from locust non-spiking interneuron. Science 204:81-83.

Bush, B.M.H. and Roberts, A. (1968). Resistance reflexes from a crab muscle receptor without impulses. Nature 218:1172-1173.

Byerly, L. and Masuda, M.O. (1979). Voltage clamp analysis of the potassium current that produces a negative-going action potential in Ascaris muscle. J. Physiol. (Lond.) 288:263-284.

Cajal, S.R. (1918). Observaciones sobre la estructura de los ocelos y vias nerviosos ocelares de algunos insectos. Trab. Lab. Invest. Biol. Univ. (Madrid) 16:109-137.

Callec, J.J. and Sattelle, D.B. (1973). A simple electro-physiological technique for monitoring the synaptic actions of pharmacological agents. J. Exp. Biol. 59:725-738.

Carew, T., Castellucci, V.F., and Kandel, E.R. (1979). Sensitization in Aplysia: Restoration of transmission in synapses inactivated by long term habituation. Science 205:417-419.

Carew, T.J. and Kandel, E.R. (1976). Two functional effects of decreased conductance EPSPS: synaptic augmentation and increased electronic coupling. Science 192:150-153.

Carpenter, D.O. (1973). Electrogenic sodium pump and high specific resistance in nerve cell bodies of the squid. Science 179: 1336-1338.

Carpenter, D.O., Swann, J.W. and Yarowsky, P.J. (1977). Effect of curare on responses to different putative neurotransmitters in Aplysia neurons. J. Neurobiol. 8:119-132.

Castellucci, V. and Kandel, E.R. (1976). Presynaptic facilitation as a mechanism for behavioral sensitization in Aplysia. Science 194:1176-1178.

Cervetto, L. and Piccolino, M. (1974). Synaptic transmission between photoreceptors and horizontal cells in the turtle retina. Science 183:417-419.

Chappell, R.L. (1970). Intracellular responses in the Anisopteran ocellus. Ph.D. Thesis, Johns Hopkins University, Baltimore, Md.

Chappell, R.L. (1979). Insect ocelli: the new look at an enigmatic eye. Ass. Res. Vis. Ophthalmol. (Abstract), p. 109.

Chappell, R.L. and Devoe, R.D. (1975). Action spectra and chromatic mechanisms of cells in the median ocelli of dragonflies. J. gen. Physiol. 65:399-419.

Chappell, R.L. and Dowling, J.E. (1972). Neural organization of the median ocellus of the dragonfly. I. Intracellular electrical activity. J. gen. Physiol. 60:121-147.

Chappell, R.L., Goodman, L.J. and Kirkham, J.B. (1978). Lateral ocellar nerve projections in the dragonfly brain. Cell Tiss. Res. 190:99-114.

Chappell, R.L. and Klingman, A.D. (1974). Synaptic feedback in the retina: a model from pharmacology of the dragonfly ocellus. Proc. Int. Union Physiol. Sci. 11:225. (Abstr.)

Chappell, R.L. and Kuhar, J. (1976). Evidence for acetylcholine in dragonfly ocellar retina. Ass. Res. Vis. Ophthalmol. (Abstract) p. 17.

Chan, P.H., Wong, Y.P. and Fishman, R.A. (1978). Hyperosmolality-induced GABA release from rat brain slices. Studies of calcium dependency and sources of release. J. Neurochem. 30:1363-1368.

Chiarandini, D.J. and Gerschenfeld, H.M. (1967). Ionic mechanism of cholinergic inhibition in molluscan neurons. Science 156: 1595-1596.

Coles, J.A. and Tsacopoulos, M. (1979). Potassium activity in photoreceptors, glial cells and extracellular space in the drone retina: changes during photostimulation. J. Physiol. (Lond.) 290:525-549.

Colguhoun, D., Dreyer, F. and Sheridan, R.E. (1979). Actions of tubocurarine at the frog neuromuscular junction. J. Physiol. (Lond.) 293:247-284.

Constanti, A. (1978). The mixed effect of picrotoxin on the GABA dose/conductance relation recorded from lobster muscle. Neuropharmacol. 17:159-168.

Cornwall, M.C. and Gorman, A.L.F. (1979). Contribution of calcium and potassium permeability changes to the off response of scallop hyperpolarizing receptors. J. Physiol. (Lond.) 291:207-232.

Curtis, D.R. and Ryall, R.W. (1966). The acetylcholine receptors of Renshaw cells. Exp. Brain Res. 2:66-80.

Curtis, D.R. and Watkins, J.C. (1965). The pharmacology of amino acids related to gamma-amino butyric acid. Pharmacol. Rev. 17:347-391.

Dale, H.H. (1914). The action of certain esters and ethers of choline, and their relation to muscarine. J. Pharmacol. 6:147-190.

Dale, H.H. (1935). Pharmacology and nerve-endings. Proc. Roy. Soc. Med. 28:319-332.

Dacheux, R.F. and Miller, R.F. (1976). Photoreceptor-bipolar cell transmission in the perfused retina eyecup of the mudpuppy. Science 171:963-964.

DeGroat, W.C. (1970). The actions of γ-aminobutyric acid and related amino acids on mammalian autonomic ganglia. J. Pharmacol. Exp. Ther. 172:384-396.

DeGroat, W.C. (1972). GABA depolarization of a sensory ganglion: antagonism by picrotoxin and bicuculline. Brain Res. 38:429-432.

DeGroat, W.C. and Lalley, P.M. (1973). Interaction between picrotoxin and 5-hydroxytryptamine in the superior cervical ganglion of the cat. Br. J. Pharmacol. 48:233-244.

DeGroat, W.C. and Simonds, W. (1976). Antagonism by picrotoxin of 5-hydroxytryptamine induced depolarization and excitation of the nodose ganglion of the cat. Neurosci. Abstr. 2:780.

Detwiler, P.B. (1976). Multiple light-evoked conductance changes in the photoreceptors of Hermissenda crassicornis. J. Physiol (Lond.) 256:691-708.

DiPolo, R. and Beauge, L. (1979). Physiological role of ATP-driven calcium pump in squid axon. Nature 278:271-273.

Dowling, J.E. (1979). Information processing by local circuits: the vertebrate retina as a model system. In: Schmitt, F.O. and Worden, F.G. (Eds.). The Neurosciences, Fourth Study Program, MIT Press, Cambridge, Mass., pp. 163-181.

Dowling, J.E. and Boycott, B.B. (1966). Organization of the primate retina: Electron microscopy. Proc. Roy. Soc. Lond. B166:80-111.

Dowling, J.E. and Chappell, R.L. (1972). Neural organization of the median ocellus of the dragonfly. II. Synaptic structure. J. gen. Physiol. 60:148-165.

Dowling, J.E. and Werblin, F.S. (1969). Organization of the retina of the mudpuppy Necturus maculosus. I. Synaptic structure. J. Neurophysiol. 32:315-338.

Duchateau, G., Florkin, M. and Leclerq, J. (1953). Concentrations de bases fixes et types de composition de la base totale de l'hemolymphe des insectes. Arch. Internat. Physiol. 61:518-549.

Duncan, C.J. and Publicover, S.J. (1979). Inhibitory effects of cholinergic agents on the release of transmitter at the frog neuromuscular junction. J. Physiol. (Lond.) 294:91-103.

Easland, G.S. and Wasserman, G.S. (1979). Multiple intracellular contributions to light adaptation in Limulus. Vision Res. 19:1-18.

Eccles, R.M. and Ubet, B. (1961). Origin and blockade of the synaptic responses of curarized sympathetic ganglia. J. Physiol. (Lond.) 157:484-503.

Eckert, R. and Lux, H.D. (1976). A voltage-sensitive persistent calcium conductance in neuronal somata of Helix. J. Physiol. (Lond.) 254:129-151.

Edgington, D.R. and Stuart, A.E. (1979). Calcium channels in the high resistivity axonal membrane of photoreceptors in the giant barnacle. J. Physiol. (Lond.) 294:433-445.

Elmquist, D. and Feldman, D.S. (1965). Effects of sodium pump inhibitors on spontaneous acetylcholine release at the neuromuscular junction. J. Physiol. (Lond.) 181:487-497.

Epstein, R. and Grundfest, H. (1970). Desensitization of gamma-aminobutyric acid (GABA) receptors in muscle fibers of the crab. Cancer borealis. J. gen. Physiol. 56:33-45.

Erber, J. and Sandeman, D.C. (1976). The detection of real and apparent motion of the crab Leptograpsus variegatus. II. Electrophysiology. J. comp. Physiol. 112:189-197.

Erulkar, S.D. and Weight, F.F. (1977). Extracellular potassium and transmitter release at the giant synapse of the squid. J. Physiol. (Lond.) 266:209-218.

Fain, G.L., Gerschenfeld, H.M. and Quandt, F.N. (1980). Calcium spikes in toad rods. J. Physiol. (Lond.) 303:495-513.

Fain, G.L. and Quandt, F.N. (1980). The effects of tetraethyl-ammonium and cobalt ions on responses to extrinsic current in toad rods. J. Physiol. (Lond.) 303:515-533.

Fain, G.L., Quandt, F.N. and Gerschenfeld, H.M. (1977). Calcium-dependent regenerative responses in rods. Nature 269:707-709.

Feltz, A. and Trautmann, A. (1980). Interaction between nerve-released acetylcholine and bath applied agonists at the frog end-plate. J. Physiol. (Lond.) 299:533-552.

Florey, E. and Murdock, L.L. (1974). The ionic mechanism of action of GABA and L-glutamate on a crustacean striated muscle (vas deferans of the crayfish). Comp. gen. Pharmacol. 5:91-99.

Freeman, A.R. (1973). Electrophysiological analysis of the actions of strychnine, bicuculline and picrotoxin on the axonal membrane. J. Neurobiol. 4:567-582.

Frumkes, T.E., Miller, R.F., Slaughter, M. and Dacheux, R.F. (1981). Physiological and pharmacological basis of GABA and glycine action on neurons of mudpuppy. III. Amacrine-mediated inhibitory influences on ganglion cell receptive-field organization: a model. J. Neurophysiol. 45:783-804.

Fulpius, B. and Bauman, F. (1969). Effects of sodium, potassium and calcium ions on slow and spike potentials in single photoreceptor cells. J. gen. Physiol. 53:541-561.

Fuortes, M.G.F. (1963). Visual responses in the eye of the dragonfly. Science 142:69-70.

Fuortes, M.G.F. and O'Bryan, P.M. (1972). Responses to single photons. In: Physiology of Photoreceptor Organs: Handbook of Sensory Physiology, Vol. VII/2, Fuortes, (Ed.). New York: Springer, pp. 321-338.

Fuortes, M.G.F., Schwartz, E.A. and Simon, E.J. (1973). Color-dependence of cone responses in the turtle retina. J. Physiol. (Lond.) 234:199-216.

Gadsby, D.C. and Cranefield, P.F. (1977). Two levels of resting potential in cardiac Purkinje fibers. J. gen. Physiol. 70: 725-746.

Gage, P.W. (1965). Effect of cardiac glycosides on neuromuscular transmission. Nature 205:84-85.

Gardner, D. and Kandel, E.R. (1972). Diphasic post-synaptic potential: A chemical synapse capable of mediating conjoint excitation and inhibition. Science 176:675-678.

Gerschenfeld, H.M. (1973). Chemical transmission in invertebrate central nervous systems and neuromuscular junctions. Physiol. Rev. 53:1-119.

Gerschenfeld, H.M. and Lasansky, A. (1964). Action of glutamic acid and other naturally occurring amino acids on snail central neurones. Int. J. Neuropharmacol. 3:301-314.

Gerschenfeld, H.M. and Paupardin-Tritsch, D. (1974a). Ionic mechanisms and receptor properties underlying the responses of molluscan neurones to 5-hydroxytryptamine. J. Physiol. (Lond.) 243:427-456.

Gerschenfeld, H.M. and Paupardin-Tritsch, D. (1974b). On the transmitter function of 5-hydroxytryptamine at excitatory and inhibitory monosynaptic junctions. J. Physiol. (Lond.) 243:457-481.

Gerschenfeld, H.M. and Piccolino, M. (1977). Muscarinic antagonists block cone to horizontal cell transmission in the turtle retina. Nature 268:257-259.

Gerschenfeld, H.M. and Piccolino, M. (1979). Pharmacology of the connections of cones and L-horizontal cells in the vertebrate retina. In: The Neurosciences, Fourth Study Program, Schmitt, F.O. and Worden, F.G. (Eds.). Cambridge, Mass: MIT Press, pp.218-219.

Gerschenfeld, H.M. and Piccolino, M. (1980). Sustained feedback effects of L-horizontal cells on turtle cones. Proc. Roy. Soc. Lond. B206:439-463.

Gillette, R., Kovac, M.P. and Davis, W.J. (1978). Command neurons in pleurobronchial receive synaptic feedback from the motor network they excite. Science 199:798-801.

Glavinovic, M.I. (1979). Presynaptic action of curare. J. Physiol. (Lond.) 290:499-506.

Goodman, C.S. (1974). Anatomy of locust ocellar interneurons: Constancy and variability. J. comp. Physiol. 95:185-201.

Goodman, C.S. (1976a). Constancy and uniqueness in a large population of small interneurons. Science 193:502-506.

Goodman, C.S. (1976b). Anatomy of the ocellar interneurons of acridid grasshoppers. I. The large interneurons. Cell Tissue Res. 175:183-202.

Goodman, C.S. and Williams, J.L.D. (1976). Anatomy of the ocellar interneurons of acridid grasshoppers. II. The small interneurons. Cell Tissue Res. 175:203-225.

Goodman, L.J. (1975). The neural organization and physiology of the insect dorsal ocellus. In: The Compound Eye and Vision of Insects, Horridge, G.A. (Ed.). Oxford: Oxford University Press.

Goodman, L.J., Mobbs, P.G. and Kirkham, J.B. (1979). The fine structure of the ocelli of Schistocerca gregaria. Cell Tissue Res. 196:487-510.

Goodman, L.J., Patterson, J.A. and Mobbs, P.G. (1975). The projection of ocellar neurons within the brain of the locust Schistocerca gregaria. Cell Tissue Res. 157:467-492.

Gorman, A.L.F. and McReynolds, J.S. (1978). Ionic effects on the membrane potential of hyperpolarizing photoreceptors in the scallop retina. J. Physiol. (Lond.) 275:345-355.

Gorman, A.L.F. and Hermann, A. (1979). Internal effects of divalent cations on potassium permeability in molluscan neurones. J. Physiol. (Lond.) 296:393-410.

Gorman, A.L.F. and Mirolli, M. (1972). The passive electrical properties of a molluscan neurone. J. Physiol. (Lond.) 227:35-49.

Graham, L.T. and Pong, S.F. (1972). Rhythmic potentials originating in the rat retina. Expl. Neurol. 36:399-403.

Graubard, K. and Calvin, W.H. (1979). Presynaptic dendrites: Implications of spikeless synaptic transmission and dendritic geometry. In: The Neurosciences, Fourth Study Program, Schmitt, F.O. and Worden, F.G. (Eds.). Cambridge, Mass: MIT Press, pp. 317-332.

Greengard, P. (1978). Phosphorylated proteins as physiological effectors. Science 199:146.

Grundfest, H., Reuben, J.P. and Rickles Jr., W.H. (1959). The electrophysiology and pharmacology of lobster neuromuscular synapses. J. gen. Physiol. 42:1301-1323.

Gwilliam, G.F. (L963). The mechanism of the shadow reflex in Cirripedia. I. Electrical activity in the supraesophageal ganglion and ocellar nerve. Biol. Bull. 125:470-485.

Gwilliam, G.F. (1965). The mechanism of the shadow reflex in Cirripedia. II. Photoreceptor cell response, second order responses, and motor cell output. Biol. Bull. 129:244-256.

Guy, R.G., Goodman, L.J. and Mobbs, P.G. (1979). Visual interneurons in the bee brain: Synaptic organization and transmission by graded potentials. J. comp. Physiol. 134:253-264.

Hagiwara, S. (1973). Calcium spikes. Adv. Biophys. 4:71-102.

Hagiwara, S. and Takahashi, K. (1967). Surface density of calcium ions and calcium spikes in barnacle muscle fiber membrane. J. gen. Physiol. 50:583-601.

Hamori, J. and Horridge, G.A. (1966). The lobster optic lamina. II. Types of synapse. J. Cell Sci. 1:257-270.

Hanani, M. and Shaw, C. (1977). A potassium contribution to the response of the barnacle photoreceptor. J. Physiol. (Lond.) 270:151-163.

Hanley, M.R., Cottrell, G.A., Emson, P.C. and Fonnum, F. (1974). Enzymatic synthesis of acetylcholine by a serotonin-containing neuron from Helix. Nature (Lond.) 251:631-633.

Hartline, H.K. and Ratliff, F. (1957). Inhibitory interaction of receptor units in the eye of Limulus. J. gen. Physiol. 40:1357-1376.

Hartline, H.K., Wagner, H.G. and MacNichol, E.F. Jr. (1952). The peripheral origin of nervous activity in the visual system. Cold Spring Harbor Symp. Quant. Biol. 17:125-141.

Hedden, W.L. and Dowling, J.E. (1978). The interplexiform cell system. II. Effects of dopamine on goldfish retinal neurons. Proc. Roy. Soc. Lond. B201:27-55.

Heldman, E., Grossman, Y., Jerrussi, T.P. and Alkon, D.L. (1979). Cholinergic features of photoreceptor synapses in Hermissenda. J. Neurophysiol. 42:153-165.

Hengstenberg, R. (1978). Spike responses of "non-spiking" visual interneurons. Nature 270:338-340.

Hill, R.G., Simmonds, M.S. and Straughan, D.W. (1976). Antagonism of γ-aminobutyric acid and glycine by convulsants in the cuneate nucleus of the cat. Brit. J. Pharmacol. 56:9-19.

Hodgkin, A.L. (1972). Recent work on visual mechanisms. (1972). Proc. Roy. Soc. Lond. B180:x-xv.

Hökfelt, L.G., Elde, E.R., Schultzberg, M., Goldstein, M. and Luft, R. (1977). Proc. Natl. Acad. Sci. USA 74:3587.

Hökfelt, T., Lundberg, J.M., Schultzberg, M., Johansson, O., Ljungdahl, A. and Rehfeld, J. (1980). Coexistence of peptides and putative transmitters in neurons. In: E. Costa and M. Trabucchi (Eds.) Neural Peptides and Neural Communication. Raven Press, New York, pp. 1-23.

Hoyle, G. (1955). Functioning of the insect ocellar nerve. J. Exp. Biol. 32:397-407.

Hudspeth, A.J., Poo, M.M. and Stuart, A.E. (1977). Passive signal propagation and membrane properties in median photoreceptors of the giant barnacle. J. Physiol.(Lond.) 272:25-42.

Hudspeth, A.J. and Stuart, A.E. (1977). Morphology and responses to light of the somata, axons and terminal regions of individual photoreceptors of the giant barnacle. J. Physiol. (Lond.) 272: 1-23.

Ioannides, A.C. and Walcott, B. (1971). Graded illumination potentials from retinula cell axons in the bug, Lethocerus. Z. Vergl. Physiol. 71:315-325.

Jack, J.J.B., Nobel, D. and Tsien, R.W. (1975). In: Electric Current Flow in Excitable Cells. Oxford: Oxford University Press, pp. 67-82, 132-224, 306-369.

Jarvilehto, M. and Zettler, F. (1970). Micro-localisation of lamino-located visual cell activities in the compound eye of the blowfly Calliphora. Z. Vergl. Physiol. 69:134-138.

Jarvilehto, M. and Zettler, F. (1971). Localized intracellular potentials from pre- and postsynaptic components in the external plexiform layer of an insect retina. Z. Vergl. Physiol. 75: 422-440.

Jenkinson, D.H. (1960). The antagonism between tubocurarine and substances which depolarize the motor end plate. J. Physiol. (Lond.) 152:309-324.

Johnston, G.A.R. (1978). Neuropharmacology of amino acid inhibitory transmitters. Ann. Rev. Pharm. 18:269-289.

Katz, B. and Thesleff, S. (1957). A study of "desensitization" produced by acetylcholine at the motor end plate. J. Physiol. (Lond.) 138:63-80.

Kandel, E.R. (1976). Cellular Basis of Behavior. San Francisco, W.H.Freeman.

Kehoe, J.S. (1972a). Ionic mechanisms of two component cholinergic inhibition in Aplysia neurons. J. Physiol. (Lond.) 225:85-114.

Kehoe, J.S. (1972b). Three acetylcholine receptors in Aplysia neurones. J. Physiol. (Lond.) 225:115-146.

Kehoe, J.S. (1972c). The physiological role of three acetylcholine receptors in synaptic transmission in Aplysia. J. Physiol. (Lond.) 225:147-172.

Kerkut, G.A. and Thomas, R.C. (1965). An electrogenic sodium pump in snail nerve cells. Comp. Biochem. Physiol. 14:167.

Kerkut, G.A. and Walker, R.J. (1961). The effect of drugs on the neurones of the snail Helix aspersa. Comp. Biochem. Physiol. 3:143-160.

Kerkut, G.A. and Walker, R.J. (1962). The specific chemical sensitivity of Helix nerve cells. Comp. Biochem. Physiol. 7:277-288.

Kirkham, J.B., Goodman, L.J. and Chappell, R.L. (1975). Identification of cobalt in process of stained neurons using X-ray energy spectra in the electron microscope. Brain Res. 85:33-37.

Kirschfield, K. (1966). Discrete and graded receptor potentials in the compound eye of the fly (Musca). In: The Functional Organization of the Compound Eye, Bernhard, G.G. (Ed.). Oxford: Pergamon Press, pp. 291-307.

Kleinschmidt, J. and Dowling, J.E. (1975). Intracellular recordings from gecko photoreceptors during light and dark adaptation. J. gen. Physiol. 66:617-648.

Klingman, A. (1976). Electrophysiological analysis of synaptic transmission in the retina of the dragonfly ocellus. Ph.D. thesis. The City University of New York, New York, N.Y.

Klingman, A. and Chappell, R.L. (1978). Feedback synaptic interactions in the dragonfly ocellar retina. J. gen. Physiol. 71:157-175.

Koelle, G.B. (1961). A proposed dual neurohumoral role of acetylcholine: Its functions at the pre- and post-synaptic sites. Nature (Lond.) 190:208-211.

Koelle, G.B. (1962). A new general concept of the neurohumoral functions of acetylcholine and acetylcholinesterase. J. Pharmac. 14:65-90.

Koelle, G.B. (1975a). Neurohumoral transmission and the autonomic nervous system. In: The Parmacological Basis of Therapeutics, 5th edition, Goodman, L.S. and Gilman, A. (Eds.). New York: Macmillan, pp. 404-444.

Koelle, G.B. (1975b). Antiacetylcholinesterase agents. In: The Pharmacological Basis of Therapeutics, 5th edition, Goodman, L.S. and Gilman, A. (Eds.). New York, Macmillan, pp. 445-466.

Koelle, G.B. (1975c). Parasympathomimetic agents. In: The Pharmacological Basis of Therapeutics, 5th edition, Goodman, L.S. and Gilman, A. (Eds.). New York: Macmillan, pp. 467-476.

Koelle, G.B. (1975d). Neuromuscular blocking agents. In: The Pharmacological Basis of Therapeutics, 5th edition, Goodman, L.S. and Gilman, A. (Eds.). New York: Macmillan, pp. 575-588.

Koike, H., Brown, H.M. and Hagiwara, S. (1970). Post-illumination hyperpolarization of a barnacle photoreceptor. Fed. Proc. 29:393.

Koike, H., Brown, H.M. and Hagiwara, S. (1971). Hyperpolarization of a barnacle photoreceptor membrane following illumination. J. gen. Physiol. 57:723-737.

Kondo, H. (1978). Efferent system of the lateral ocellus in the dragonfly: Its relationships with the ocellar afferent units, the compound eyes, and the wing sensory system. J. comp. Physiol. (A)125:341-349.

Krnjevic, K. (1974). Chemical nature of synaptic transmission in vertebrates. Physiol. Rev. 54:418-539.

Krnjevic, K., Pumain, R. and Renaud, J. (1971). The mechanism of excitation by acetylcholine cerebral cortex. J. Physiol. (Lond.) 215:247-268.

Krnjevic, K., Randic, M. and Straughan, D.W. (1966). Pharmacology of cortical inhibition. J. Physiol. (Lond.) 184:78-103.

Kuba, K. (1980). Release of calcium ions linked to the activation of a potassium conductance in a caffeine-treated sympathetic neuron. J. Physiol. (Lond.) 298:251-269.

Kupferman, I. (1979). Modulatory actions of neurotransmitters. Ann. Rev. Neurosci. 2:447-465.

Lam, D.M.K., Lasiter, E.M. and Naka, K.J. (1979). Gamma-aminobutyric acid: Neurotransmitter candidate for cone horizontal cells of the catfish retina. Proc. Natl. Acad. Sci. 75(12):6310-6313.

Lam, D.M.K., Su, Y.Y.T., Swain, L., Marc, R.E., Brandon, C. and Wu, J.Y. (1979). Immunocytochemical localization of L-glutamic acid decarboxylase in the goldfish retina. Nature 278:565.

Lang, G.D. and Hartline, P.H. (1974). Retinal responses in squid and octopus. J. comp. Physiol. 93:19-36.

Langer, G.A. and Serena, S.D. (1970). Effects of strophanthidin upon contraction and ionic exchange in rabbit ventricular myocardium: Relation to control of active state. J. Mol. Cell. Cardiol. 1:65-90.

Laughlin, S.B. (1973). Neural integration in the first optic neuropile of dragonflies. I. Signal amplification in dark-adapted second order neurons. J. comp. Physiol. 84:335-355.

Laughlin, S.B. (1974a). Neural integration in the first optic neuropile of dragonflies. II. Receptor signal interactions in the lamina. J. comp. Physiol. 92:357-375.

Laughlin, S.B. (1974b). Resistance changes associated with the response of insect monopolar neurons. Z. Naturforsch 29c: 449-450.

Laughlin, S.B. and Hardie, R.C. (1978). Common strategies for light adaptation in the peripheral visual systems of fly and dragonfly. J. comp. Physiol. 128:319-340.

Leonard, R.J. and Lisman, J.E. (1981). Light modulates voltage-dependent potassium channels in Limulus ventral photoreceptors. Science 212:1273-1275.

Levy, R.A. (1974). GABA-A direct depolarising action at the mammalian primary afferent terminal. Brain Res. 76:155-156.

Levy, R. (1977). The role of GABA in primary afferent depolarization. Prog. Neurobiol. 9:211-267.

Lillywhite, P.G. (1977). Single photon signals and transduction in an insect eye. J. comp. Physiol. 122:189-200.

Lillywhite, P.G. (1978). Coupling between locust photoreceptors revealed by a study of quantum bumps. J. comp. Physiol. A125:13-27.

Lillywhite, P.G. and Laughlin, S.B. (1979). Transducer noise in a photoreceptor. Nature 277:569-572.

Lisman, J.E. and Brown, J.E. (1971). Two light-induced processes in the photoreceptor cells in Limulus ventral eye. J. gen. Physiol. 58:544-561.

Lux, H.D. and Heyer, C.B. (1979). A new electrogenic calcium-potassium system. In: The Neurosciences Fourth Study Program, Schmitt, F.O. and Worden, F.G. (Eds.). Cambridge, Mass.: MIT Press, pp. 601-615.

MacNichol, E.F. Jr. (1958). Visual receptors as biological transducers. In: Molecular Structure and Functional Activity of Nerve Cells, Grenell, R.G. and Mullins, L.J. (Eds.). Washington, D.C.: Ann. Inst. Biol. Sci., pp. 34-62.

MacNichol, E. and Svaetichin, G. (1958). Electrical responses from the isolated retinas of fishes. Am. J. Ophthal. 46:26-40.

Manalis, R.S. (1977). Voltage-dependent effects of curare at the frog neuromuscular junction. Nature (Lond.) 267:360-366.

Marc, R.E., Stell, W.K., Bok, D. and Lam, D.M.K. (1978). GABA-ergic pathways in the goldfish retina. J. comp. Neurol. 182:221-245.

Marchais, D. and Marty, A. (1979). Interaction of permeant ions with channels activated by acetylcholine in Aplysia neurons. J. Physiol. (Lond.) 297:9-45.

Marder, E. and Paupardin-Tritsch, D. (1978). The pharmacological properties of some crustacean neuronal acetylcholine, γ-amino-butyric acid, and L-glutamic responses. J. Physiol. (Lond.) 280:213-236.

Marder, E. and Paupardin-Tritsch, D. (1980). Picrotoxin block of a depolarizing ACh response. Brain Res. 181:223-227.

Marshall, K.C. and Engberg, I. (1979). Reversal potential for nonadrenaline-induced hyperpolarization of spinal motor-neurons. Science 205:422-424.

Marty, N., Neild, T.O. and Ascher, P. (1976). Voltage sensitivity of acetylcholine currents in Aplysia neurons in the presence of curare. Nature 261:501-503.

McReynolds, J.S. and Gorman, A.L.F. (1970). Membrane conductance and spectral sensitivities of Picten photoreceptors. J. gen. Physiol. 56:392-406.

Meech, R.W. (1972). Intracellular calcium injection causes increased potassium conductance in Aplysia nerve cells. Comp. Biochem. Physiol. 42A:493-499.

Meech, R.W. (1976). Intracellular calcium and the control of membrane permeability. In: Calcium in Biological Systems, Symp. Soc. Exp. Biol. No. 30. Cambridge University Press: Cambridge, pp. 161-191.

Mendelson, M. (1971). Oscillator neurons in crustacean ganglia. Science 171:1170-1173.

Menzel, R. (1974). Spectral sensitivity of monopolar cells in the bee lamina. J. comp. Physiol. 93:334-337.

Miledi, F.R.S. (1973). Transmitter release induced by injection of calcium ions into nerve terminals. Proc. Roy. Soc. Lond. B183: 421-425.

Millecchia, R. and Gwilliam, G.F. (1972). Photoreception in a barnacle: Electrophysiology of the shadow reflex pathway in Balanus cariosus. Science 177:438-441.

Millecchia, R. and Mauro, A. (1969a). The ventral photoreceptor cells of Limulus. II. The basic photo response. J. gen. Physiol. 54: 310-330.

Millecchia, R. and Mauro, A. (1969b). The ventral photo receptor cells of Limulus. III. A voltage clamp study. J. gen. Physiol. 54:331-351.

Miller, R.F., Frumkes, T.E., Slaughter, M. and Dacheux, R.F. (1981a). Physiological and pharmacological basis of GABA and Glycine Action on Neurons of Mudpuppy retina. I. Receptors, Horizontal cells, bipolars, and G-cells. J. Neurophysiol. 45:743-763.

Miller, R.F., Frumkes, T.E., Slaughter, M. and Dacheux, R.F. (1981b). Physiological and pharmacological basis of GABA and glycine action on neurons in the mudpuppy retina. II. Amacrine and ganglion cells. J. Neurophysiol. 45:764-782.

Miyamoto, M.D. (1978). The actions of cholinergic drugs on motor terminals. Pharmac. Rev. 29:221-247.

Miyamoto, M.D. and Volle, R.L. (1974). Enhancement by carbachol of transmitter release from motor nerve terminals. Proc. Natl. Acad. Sci. USA 71:1489-1492.

Mobbs, P.G., Guy, R.G., Goodman, L.J. and Chappell, R.L. (1981). Spectral sensitivity and reverse Purkinje shift in identified L-neurons of the ocellar retina. J. comp. Physiol. 144:91-97.

Muijser, H. (1979). The receptor potential of retinula cells of the blowfly Calliphora: The role of sodium, potassium and calcium ions. J. comp. Physiol. 132:87-95.

Murakami, M., Shimoda, Y., Nakatani, K. (1978). Effects of GABA on neuronal activities in the distal retina of the carp. Sensory Proc. 2:334-338.

Naka, K. (1961). Recording of retinal action potentials from single cells in the insect compound eye. J. gen. Physiol. 44:571.

Naka, K. and Eguchi, E. (1962). Spike potentials recorded from the insect photoreceptor. J. gen. Physiol. 45:663-680.

Negishi, K. and Drujan, B.D. (1979). Effects of some amino acids on horizontal cells in the fish retina. Journal of Neurosci. Res. 4:352-363.

Nelson, R. (1973). A comparison of electrical properties of neurons in Necturus retina. J. Neurophysiol. 35:519-535.

Nelson, R., Lutzow, A.V., Kolb, H. and Gouras, P. (1975). Horizontal cells in the cat retina with independent dendritic systems. Science 189:137-139.

Norman, R.A. and Pochobradsky, J. (1976). Oscillation in rod and horizontal cell membrane potential: Evidence for feedback to rods in the vertebrate retina. J. Physiol. 261:15.

Obata, K. (1976). Excitatory effects of GABA. In: GABA in Nervous System Function, Roberts, E., Chase, T.N., and Tower, D.B. (Eds.). New York: Raven Press, pp. 283-286.

O'Donohue, T.L., Handelman, G.E., Miller, R.L. and Jacobowitz, D.M. (1982). N-acetylation regulates the behavioral activity of α-melanotropin in a multineurotransmitter neuron. Science 215:1125-1127.

Oertel, E. and Stuart, A.E. (1981). Transformation of signals by interneurones in the barnacle's visual pathway. J. Physiol. (Lond.) 311:127-146.

Ogden, T. (1973). The oscillatory waves of the primate electroretinogram. Vision Res. 13:1059-1074.

Olsen, R.W., Lee, J.M. and Ban, M. (1975). Bending of γ-aminobutyric acid to crayfish muscle sites possibly related to γ-aminobutyric acid receptor-ionophores.

Olsen, R.W., Ticku, M.K. and Miller, T. (1978). Dihydropicrotoxin binding to crayfish muscle sites possibly related to γ-aminobutyric acid receptor ionophores. Molec. Pharmacol. 14:381-390.

Oomura, Y., Sawada, M., Tanikawa, T. and Ooyama, H. (1974). Depolarization of Onchidium neurone by glycine. Nature 250:258-260.

Ozawa, S., Hagawara, S., Nicolaysen, K. and Stuart, A.E. (1975). Signal transmission from photoreceptors to ganglion cells in the visual system of the giant barnacle. Cold Spring Harbor Symp. Quant. Biol. 40:563-570.

Pan, K.C. and Goodman, L.J. (1977). Ocellar projections within the central nervous system of the worker honey bee, A pro melligera. Cell Tiss. Res. 176.505-527.

Paton, W.D.M. and Perry, W.L.M. (1953). The relationship between depolarization and block in the cat's superior cervical ganglion. J. Physiol. (Lond.) 119:43-47.

Patterson, J.A. and Chappell, R.L. (1980). Intracellular responses of procion filled cells and whole nerve cobalt impregnation in the dragonfly median ocellus. J. comp. Physiol. 139:25-39.

Patterson, J.A. and Goodman, L.J. (1974a). Intracellular responses of receptor cells and second order cells in the ocelli of the desert locust Shistocerca gregaria. J. comp. Physiol. 95:237-250.

Patterson, J.A. and Goodman, L.J. (1974b). Relationships between ocellar units in the ventral nerve cord and ocellar pathways of Schistocerca gregaria. J. comp. Physiol. 95:251-262.

Pearson, K.G. (1979). Local neurons and local interactions in the nervous system of invertebrates. In: The Neurosciences Fourth Study Program, Schmitt, F.O. and Warden, F.G. (Eds.). Cambridge, Mass.: MIT Press, pp. 145-157.

Pearson, K.G. and Fourtner, C.R. (1975). Non-spiking interneurons in the walking system of the cockroach. J. Neurophysiol. 38:33-52.

Pellmar, T.C. and Wilson, W.A. (1977). Unconventional serotonergic excitation in Aplysia. Nature 269:76-78.

Piccolino, M. and Gerschenfeld, H.M. (1977). Lateral interactions in the outer plexiform layer of turtle retinas after atropine block of horizontal cells. Nature 268:259-261.

Piccolino, M. and Gerschenfeld, H.M. (1980). Characteristics and ionic processes involved in feedback spikes of turtle cones. Proc. Roy. Soc. Lond. B206:439-463.

Pickles, H.G. (1979). Presynaptic γ-aminobutyric acid responses in the olfactory cortex. Br. J. Pharmacol. 65:223-228.

Pinsker, H. and Kandel, E.R. (1969). Synaptic activation of an electrogenic sodium pump. Science 163:931-935.

Pinto, L.H. and Pak, W.L. (1974). Light-induced changes in photoreceptor membrane resistance and potentials in gecko retinas. II. Preparations with active lateral interactions. J. gen. Physiol. 64:49-69.

Rall, W. (1959). Dendritic current distribution and whole neuron properties. Naval Med. Res. Inst., Research Report NM01-05-00.01.02.

Raper, J.A. (1979). Nonimpulse-mediated synaptic transmission during the generation of a cyclic motor program. Science 205:304-306.

Richhardt, H.W., Knauf, P.N. and Fuhrmann, G.F. (1979). Dog red blood cells exhibit a Ca-stimulated increase in K permeability in the absence of (Na, K)ATPase activity. Nature 279:248-250.

Ripley, S.H., Bush, M.H. and Roberts, A. (1968). Crab muscle receptor which responds without impulses. Nature 218:1170-1171.

Ross, W.N. and Stuart, A.E. (1978). Voltage sensitive calcium channels in the presynaptic terminals of a decrementally conducting photoreceptor. J. Physiol. (Lond.) 274:173-191.

Rosser, B.L. (1974). A study of the afferent pathways of the dragonfly lateral ocellus from intracellularly recorded spike discharges. J. Exp. Biol. 60:135-160.

Ruck, P. (1957). The electrical responses of dorsal ocelli in cockroaches and grasshoppers. J. Insect Physiol. 1:109-123.

Ruck, P. (1961a). Electrophysiology of the insect dorsal ocellus. I. Origin of the components of the electroretinogram. J. gen. Physiol. 44:605-627.

Ruck, P. (1961b). Electrophysiology of the insect dorsal ocellus. II. Mechanisms of generation and inhibition of impulses in the ocellar nerve of dragonflies. J. gen. Physiol. 44:629-639.

Ruck, P. (1961c). Electrophysiology of the insect dorsal ocellus. III. Responses to flickering light of the dragonfly ocellus. J. gen. Physiol. 44:641-657.

Ruck, P. and Edwards, G. (1964). The structure of the insect dorsal ocellus. I. General organization of the ocellus in dragonflies. J. Morphol. 115:1-26.

Russel, D.F. and Hartline, D.K. (1978). Bursting in neural networks: A re-examination. Science 200:453-456.

Sattelle, D.B., McClay, A.S., Dowson, R.J. and Callec, J.J. (1976). The pharmacology of an insect ganglion: Actions of carbamylcholine and acetylcholine. J. Exp. Biol. 64:13-23.

Schmitt, F.O., Dev, P., and Smith, B.H. (1976). Electronic processing of information by brain cells. Science 193:114-120.

Scholes, J.H. (1964). Discrete subthreshold potentials from the dimly lit insect eye. Nature (Lond.) 202:572-573.

Scholes, J. (1969). The electrical responses of the retinal receptors and the lamina in the visual system of the fly Musca. Kybernetik 6:149-162.

Schulman, J.A. and Weight, F.F. (1976). Synaptic transmission: Long-lasting potentiation by postsynaptic mechanism. Science 194: 1437-1439.

Shaw, S.R. (1968). Organization of the locust retina. Symp. Zool. Soc. Lond. 23:135-163.

Shaw, S.R. (1972). Decremental conduction of the visual signal in the barnacle lateral eye. J. Physiol. (Lond.) 220:145-173.

Shaw, S.R. (1975). Retinal resistance barriers and electrical lateral inhibition. Nature 255:480-483.

Shaw, S.R. (1977). Restricted diffusion and extracellular space in the insect retina. J. comp. Physiol. 113:257-282.

Shaw, S.R. (1978). The extracellular space and blood eye barrier in an insect retina: An ultrastructural study. Cell Tissue Res. 188:35-61.

Shaw, S. (1979). Signal transmission by graded slow potentials in the arthropod peripheral visual system. In: The Neurosciences, Fourth Study Program, Schmitt, F.O. and Worden, F.G. (Eds.). Cambridge, Mass.: MIT Press, pp. 275-295.

Shepherd, G.M. (1979). The Synaptic Organization of the Brain, 2nd ed., New York: Oxford University Press.

Simmonds, M.A. (1978). Presynaptic actions of γ-aminobutyric acid and some antagonists in a slice preparation of cuneate nucleus. Br. J. Pharmacol. 63:495-502.

Simon, Elliot J. (1974). Feedback loop between cones and horizontal cells in the turtle retina. Fed. Proc. 33:1078-1082.

Skou, J.C. (1965). Enzymatic basis for active transport of Na^+ and K^+ across cell membranes. Physiol. Rev. 45:596-617.

Slaughter, M.M. and Miller, R.F. (1981). 2-amino-4-phosphobutyric acid: A new pharmacological tool for retina research. Science 211:182-184.

Smith, P.A. and Weight, F.F. (1977). Role of electrogenic sodium pump in slow synaptic inhibition is re-evaluated. Nature 267:68-70.

Smith, T.G. and Bauman, F. (1969). The functional organization within the ommatidium of the lateral eye of Limulus. In: Progress in Brain Research, Vol. 31, Akert, K. and Waser, P.G. (Eds.). Amsterdam: Elsevier.

Smith, T.G., Stell, W.K., Brown, J.E., Freeman, J.A. and Murray, G.C. (1968). A role for the sodium pump in photoreception in Limulus. Science 162:456.

Stone, S.L. and Chappell, R.L. (1979). Receptor off-response modified by synaptic blocking agents. Ass. Res. Vis. Ophthal. (Abstract), p. 32.

Strausfeld, N.J. and Campos-Ortega, J.A. (1977). Vision in insects: Pathways possibly underlying neural adaptation and lateral inhibition. Science 195:894-897.

Stuart, A.E. and Oertel, D. (1978). Neuronal properties underlying processing of visual information in the barnacle. Nature 275:287-290.

Svaetichin, G. (1953). The cone action potential. Acta. Physiol. Scand. 29 (Suppl.) 106:565-599.

Svenneby, G. and Roberts, E. (1973). Bicuculline and N-methyl bicuculline: Competitive inhibitors of brain acetylcholinesterase. J. Neurochem. 21:1025-1026.

Svenneby, G. and Roberts, E. (1974). Elevated acetylcholine contents in mouse brain after treatment with bicuculline and picrotoxin. J. Neurochem. 23:275-277.

Takeuchi, A. (1976). Studies of inhibitory effects of GABA in invertebrate nervous systems. In: GABA In Nervous System Function, Roberts, E. and Tower, D.B. (Eds.). New York: Raven Press, pp. 255-267.

Takeuchi, A. and Takeuchi, N. (1969). A study of the action of picrotoxin on the inhibitory neuromuscular junction of the crayfish. J. Physiol. (Lond.) 205:377-391.

Tauc, L. and Gerschenfeld, H.M. (1961). Cholinergic transmission mechanism for both excitation and inhibition in molluscan central synapse. Nature 192:366-367.

Tauc, L. and Gerschenfeld, H.M. (1962). A cholinergic mechanism of inhibitory synaptic transmission in a molluscan nervous system. J. Neurophysiol. 25:236-262.

Thibos, L.N. and Werblin, F.S. (1978). The response properties of the steady antagonistic surround in the mudpuppy retina. J. Physiol. (Lond.) 278:79-99.

Thomas, R.C. (1972). Electrogenic sodium pump in nerve and muscle cells. Physiol. Rev. 220:55-71.

Thompson, S.H. (1977). Three pharmacologically distinct potassium channels in molluscan neurones. J. Physiol. (Lond.) 265:465-488.

Ticku, M.K. and Olsen, R.W. (1977). γ-Amino-butyric acid-stimulated chloride permeability in crayfish muscle. Biochim. Biophys. Acta. (Amst.) 464:519-529.

Toh, Y. and Kuwabara, M. (1974). Fine structure of the dorsal ocellus of the worker honeybee. J. Morphol. 143:285-306.

Tomita, T. (1965). Electrophysiological study of the mechanisms subserving color coding in the fish retina. Cold Spring Harbor Symp. Quant. Biol. 30:559-566.

Tomita, T., Kaneko, A., Murakami, M. and Pautler, E.L. (1967). Spectral response curves of single cones in the carp. Vision Res. 7:519-531.

Toyoda, J. (1973). Membrane resistance changes underlying the bipolar cell response in the carp retina. Vision Res. 13:283-314.

Toyoda, J., Nosaki, H. and Tomita, T. (1969). Light induced resistance changes in single photoreceptors of Necturus and Gekko. Vision Res. 9:453-463.

Trifonov, Y.A., Byzov, A.L. and Chailahian, L.M. (1974). Electrical properties of subsynaptic and non-synaptic membranes of horizontal cells in fish retina. Vision Res. 14:229-241.

Trujillo-Cenoz, O. (1965). Some aspects of the structural organization of the arthropod eye. Cold Spring Harbor Symp. Quant. Biol. 30:371-389.

Van der Kloot, W.G. and Cohen, I. (1979). Membrane surface potential changes may alter drug interactions: An example, acetylcholine and curare. Science 203:1351-1353.

Volle, R.L. and Koelle, G.B. (1975). Ganglionic stimulating and blocking agents. In: The Pharmacological Basis of Therapeutics, 5th ed., Goodman, L.S. and Gilman, A. (Eds.). New York: Macmillan, pp. 565-574.

Wachmeister, L. and Dowling, J.E. (1978). The oscillatory potentials of the mudpuppy retina. Invest. Ophthalmol. Vis. Sci. 17: 1176-1188.

Walker, R.J., Azanza, M.J., Kerkut, G.A. and Woodruff, G.N. (1975). The action of γ-aminobutyric acid (GABA) and related compounds on two identifiable neurons in the brain of the snail Helix aspersa. Comp. Biochem. Physiol. 50C:147-154.

Weakly, J.N. (1973). The action of cobalt ions on neuromuscular transmission in the frog. J. Physiol. (Lond.) 234:597-612.

Weight, F.F. and Votova, J. (1970). Slow, synaptic excitation in sympathetic ganglion cells: Evidence for synaptic inactivation of potassium conductance. Science 170:755-758.

Weiler, R. and Zettler, F. (1979). The axon-bearing horizontal cells in the teleost retina are functional as well as structural units. Vision Res. 19:1261-1268.

Werblin, F.S. (1972). Lateral interactions at the inner plexiform layer of the retina: Antagonistic response to change. Science 175:1008-1010.

Werblin, F.S. (1974). Control of retinal sensitivity. II. Lateral interactions at the outer plexiform layer. J. gen. Physiol. 63:82-87.

Werblin, F.S. (1975). Regenerative hyperpolarization in rods. J. Physiol. (Lond.) 244:53-81.

Werblin, F.S. and Copenhagen, (1974). Control of retinal sensitivity. III. Lateral interactions at the inner plexiform layer. J. gen. Physiol. 63:88-110.

Werblin, F.S. and Dowling, J.E. (1969). Organization of the retina of the mudpuppy Necturus maculosis. II. Intracellular recording. J. Neurophysiol. 32:339-355.

Whitehead, R. and Purple, R.L. (1970). Synaptic organization in the neuropil of the lateral eye of Limulus. Vision Res. 10:129-133.

Wilson, M. (1978a). The functional organization of locust ocelli. J. comp. Physiol. 124:297-316.

Wilson, M. (1978b). Generation of graded potential signals in the second order cells of locust ocellus. J. comp. Physiol. 124:317-331.

Wilson, M. (1978c). The origin and properties of discrete hyperpolarizing potentials in the second order cells of the locust ocellus. J. comp. Physiol. 128:347-358.

Wilson, W.A. and Wachtel, H. (1978). Prolonged inhibition in burst firing neurons: Synaptic inactivation of the slow regenerative inward current. Science 202:772-775.

Wu, C.F. and Pak, W.L. (1975). Quantal basis of photoreceptor spectra sensitivity of Drosophila melanogaster. J. gen. Physiol. 66: 149-168.

Wu, S.M. and Dowling, J.E. (1979). Effects of GABA and glycine on the cyprinid retina. Ass. Res. Vis. Ophthalmol. (Abstract), p. 33.

Yarowsky, P.J. and Carpenter, D.O. (1977). GABA mediated excitatory responses on Aplysia neurons. Life Science 20:1441-1448.

Yarowsky, P.J. and Carpenter, D.O. (1978). A comparison of similar ionic responses to γ-aminobutyric acid and acetylcholine. J. Neurophysiol. 41:531-541.

Yeandle, S. (1958). Electrophysiology of the visual system. Discussion. Amer. J. Ophthalmol. 46:82-87.

Zettler, F. and Autrum, H. (1975). Chromatic properties of lateral inhibition in the eye of a fly. J. comp. Physiol. 97:181-188.

Zettler, F. and Jarvilehto, M. (1971). Decrement-free conduction of graded potentials along the axon of a monopolar neuron. Z. Vergl. Physiol. 75:402-421.

Zettler, F. and Jarvilehto, M. (1972). Intra-axonal visual responses from visual cells and second order neurons of an insect retina. In: Information Processing in the Visual Systems of Arthropods, Wehner, R. (Ed.). Berlin-Heidelberg-New York: Springer, pp. 217-222.

Zettler, F. and Jarvilehto, M. (1973). Active and passive axonal propagation of nonspike signals in the retina of Calliphora. J. comp. Physiol. 85:89-104.

Zettler, F. and Weiler, R. (1976). Neuronal processing of the first optic neuropil of the compound eye of the fly. In: Neural Principles in Vision, Zettler, F. and Weiler, R. (Eds.). Berlin-Heidelberg-New York: Springer-Verlag, pp. 227-237.

Zimmerman, R.P. (1978). Field potential analysis and the physiology of the second-order neurons in the visual system of the fly. J. comp. Physiol. 126:297-316.

Figure 1

Schematic diagram showing dragonfly median ocellus and brain. (r): rhabdomeric region of receptor layer; (n): nuclear region of receptor layer; (s): synaptic region of ocellus; (ON): ocellar nerve. (Modified from Patterson and Chappell, 1980).

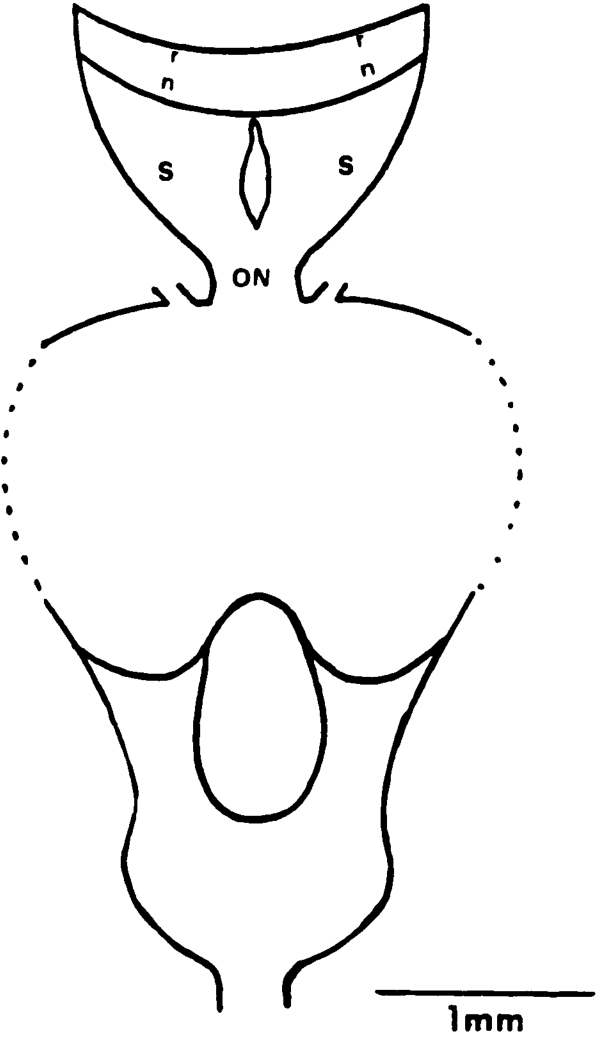


Figure 1

Figure 2

Intensity-response series showing typical photoreceptor responses from an intact nerve preparation. The single spike at light-ON is commonly observed in the dragonfly. Note the hyperpolarizing transient following light-OFF which is especially prominent at lower light intensities and least pronounced following the brightest (Log I = 0) stimulus. This cell displayed small, light-inhibited oscillations in the dark which were present in approximately 15% of the cells examined and were considered to be within the normal range of receptor responses. Such oscillations are thought to be significant because they can be modified by drugs and stimulus conditions. Stimulus intensity increases from top to bottom.
In this figure, and all subsequent figures, the duration of the light stimulus is indicated by a horizontal bar or square wave at the bottom of each record. The data in this figure is represented in graphs I and II.
Resting potential: -45 mv.

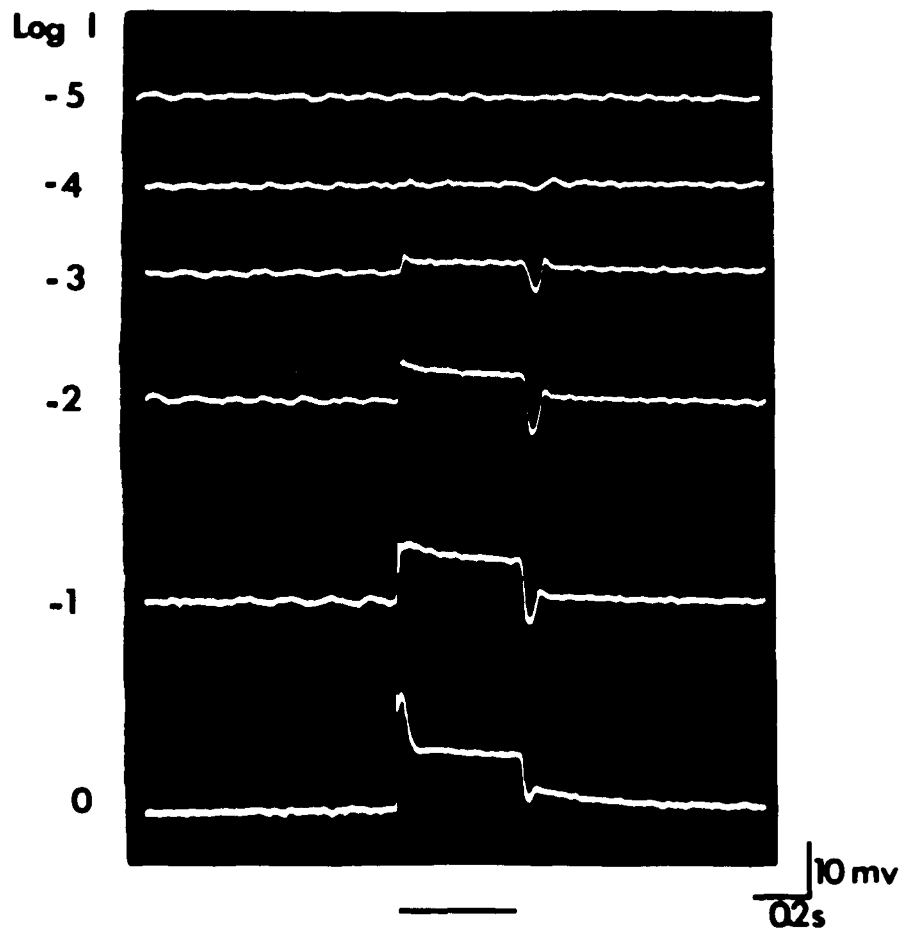


Figure 2

Figure 3

Intensity-response series showing typical photoreceptor responses from a different intact nerve preparation. In this cell, the ON-spike was less pronounced than the ON-spike in figure 2 and no dark oscillatory activity was observed. The OFF-hyperpolarization was most prominent following lower light intensities and was not observed following an unattentuated (Log I = 0) test flash. These responses were recorded from a preparation that had been washing in Ringer solution for 3.5 hours. Stimulus intensity increases from top to bottom. The data in this figure is represented in graphs I and II.

Resting potential: -40 mv.

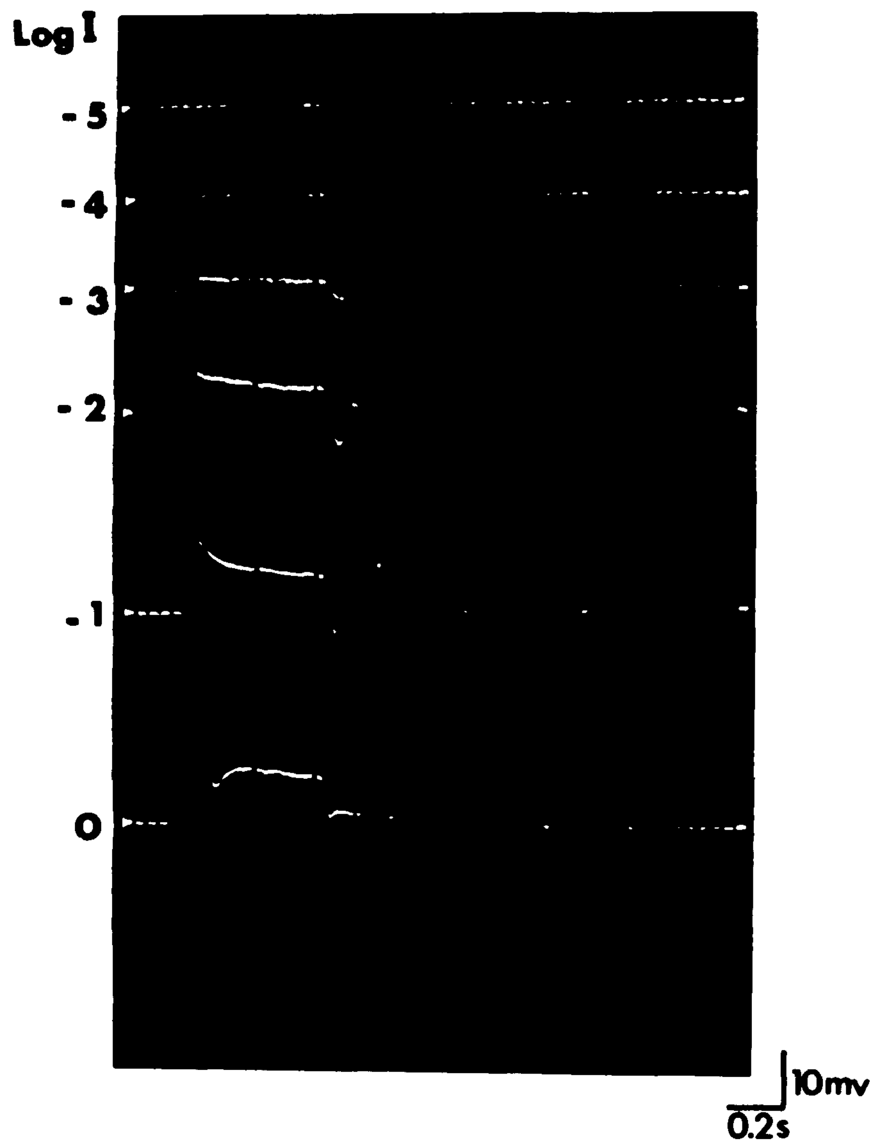


Figure 3

Figure 4

Photoreceptor responses recorded from two different sites in the same intact nerve preparation. The responses from both cells were recorded under identical conditions of illumination. The distally impaled cell (A) showed a larger than usual light-evoked depolarization and no OFF response. Note the noisy voltage fluctuations and small discrete depolarizing potentials when the recording micro-electrode is closer to the rhabdomeric end of the retinula cell. When the micro-electrode was repositioned approximately .1 mm more proximally, closer to the nuclear layer and receptor terminal (B), the more typical light response and OFF-transient were observed. Stimulus intensity increases from bottom to top. Resting potentials: distally recorded cell, -80 mv; proximally recorded cell: -53 mv.

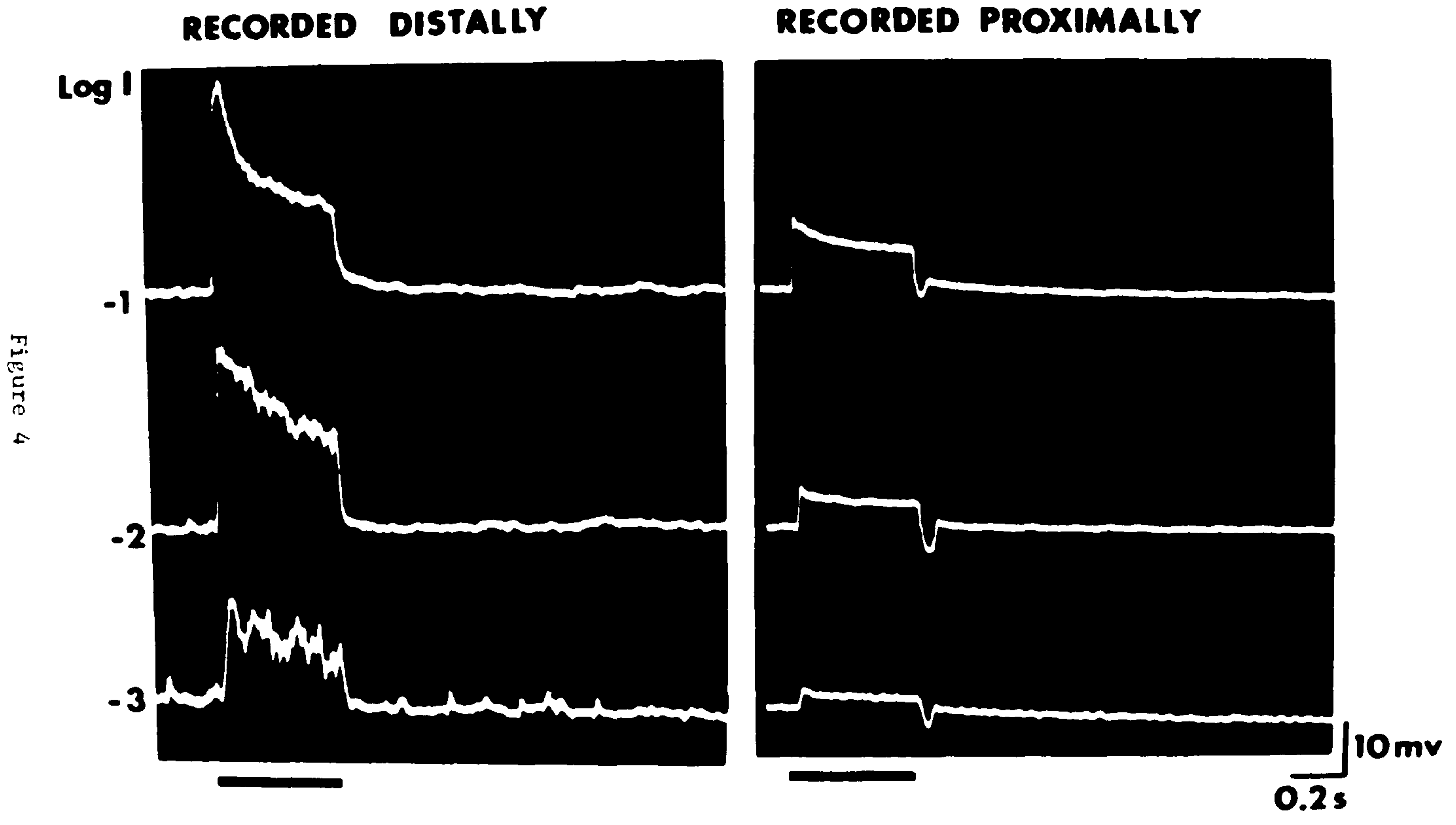


Figure 4

Figure 5

Intensity-response series from presumed receptor terminal in an intact nerve preparation. This cell was impaled in the synaptic region of the ocellus, just after recording from the second order neuron shown in figure 17 (A). The waveform of the light response and OFF-transient are similar to the typical photoreceptor response recorded more proximally. At higher light intensities, the ON-transient portion of the light response is larger relative to the sustained portion of the response. Stimulus intensity increases from top to bottom.
Resting potential: -53 mv.

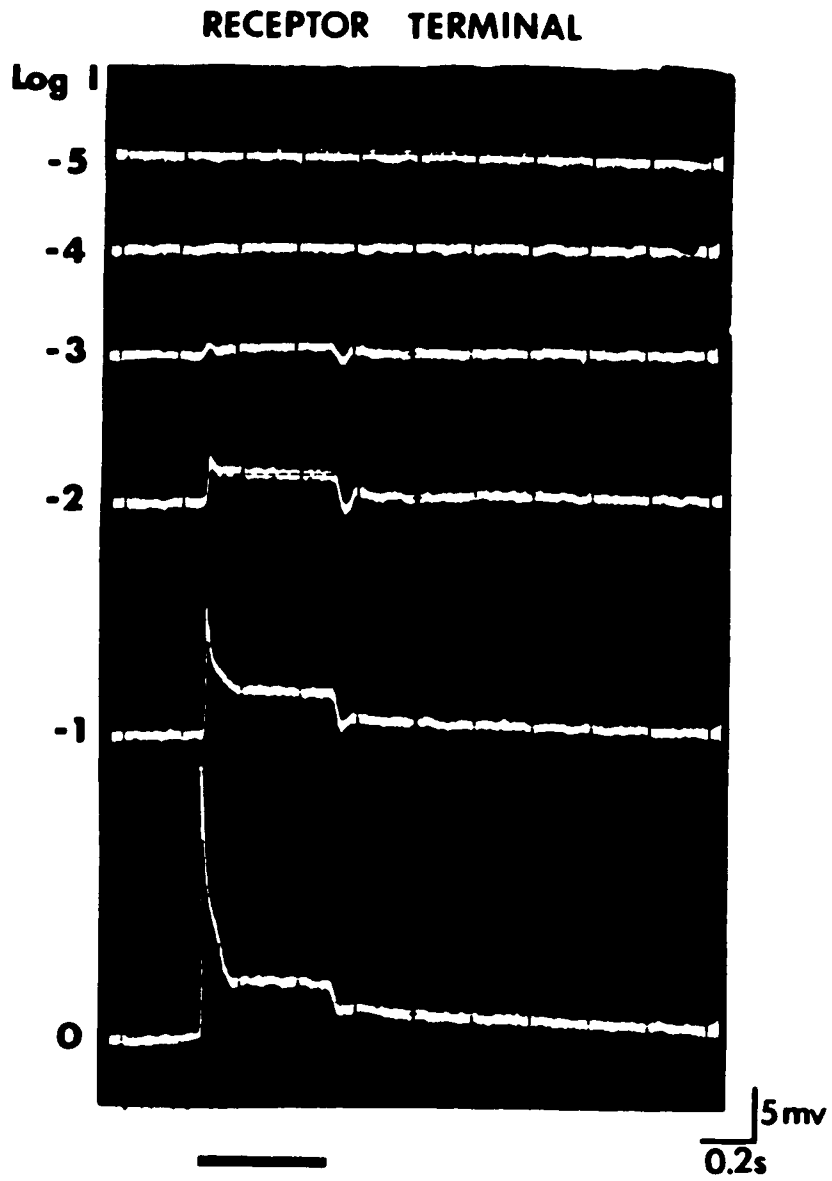


Figure 5

Figure 6

Resistance changes recorded from the distally impaled photoreceptor shown in figure 4. The light-evoked depolarization was accompanied by a large decrease in resistance. Resistance changes were measured by passing 0.2 nanoamp depolarizing pulses through a bridge circuit, where a downward deflection of the pulse-ON portion of the trace represents a resistance decrease. In this experiment, a deflection of 1 mv is approximately equivalent to a resistance change of 5 megohms, which corresponds to a decrease in input resistance of approximately 30 megohms during illumination.
Stimulus intensity: Log I = -1.

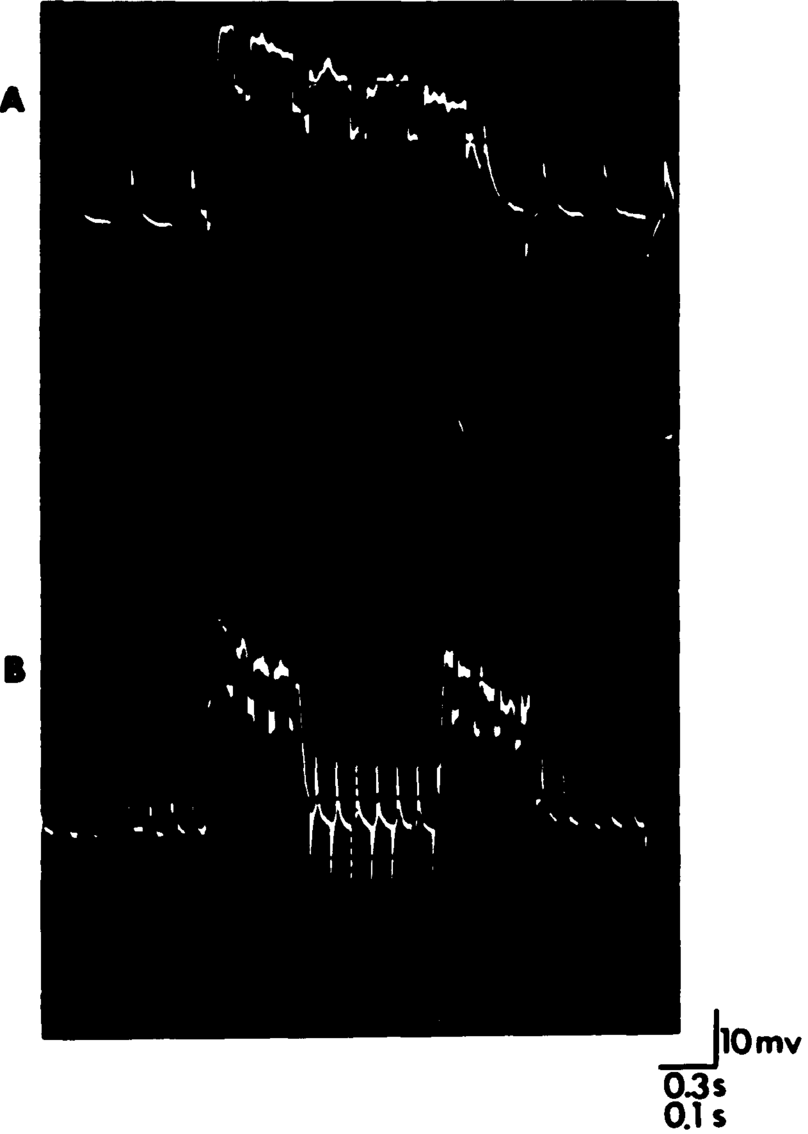


Figure 6

Figure 7

Resistance changes recorded from photoreceptors impaled in the more proximal photoreceptor region. These responses were recorded from two different intact nerve preparations. The light response was accompanied by a resistance decrease which was smaller than that observed during the distally recorded response. Resistance changes were measured by passing 1.0 nanoamp depolarizing pulses through a bridge circuit. A downward deflection of the pulse-ON portion of the trace represents a resistance decrease, with a 1 mv deflection approximately equivalent to 1 megohm, which corresponds to approximately 1-3 megohm decrease in input resistance during illumination. Response in B was recorded from a preparation that had been washing in Ringer solution almost 4 hours. This cell showed periodic increases and decreases in resistance in the dark which were not accompanied by a change in voltage. A resistance change following light OFF was not a consistent observation in the photoreceptor response.

Stimulus intensity: A, Log I = -2; B, Log I = 0.

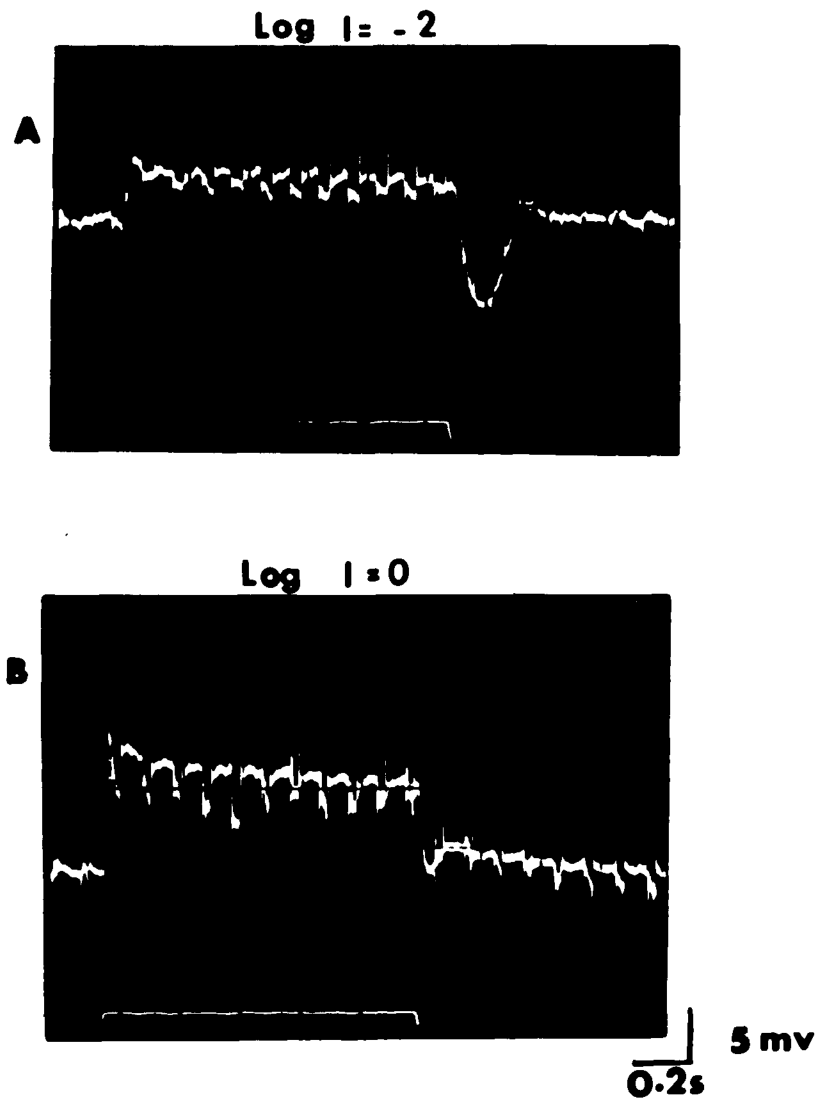


Figure 7

Figure 8

Resistance changes recorded from receptor terminal. The light-evoked depolarization was associated with a resistance decrease which was measured by passing 1.0 nanoamp depolarizing pulses through a bridge circuit. A downward deflection of the pulse-ON portion of the trace represents a resistance decrease, with a 1 mv deflection approximately equivalent to 1 megohm. During illumination the input resistance decreased approximately 2 megohms. Stimulus intensity (preparation dark adapted for 1 minute): upper, Log I = 0; lower, Log I = -1. Resting potential: -53 mv.

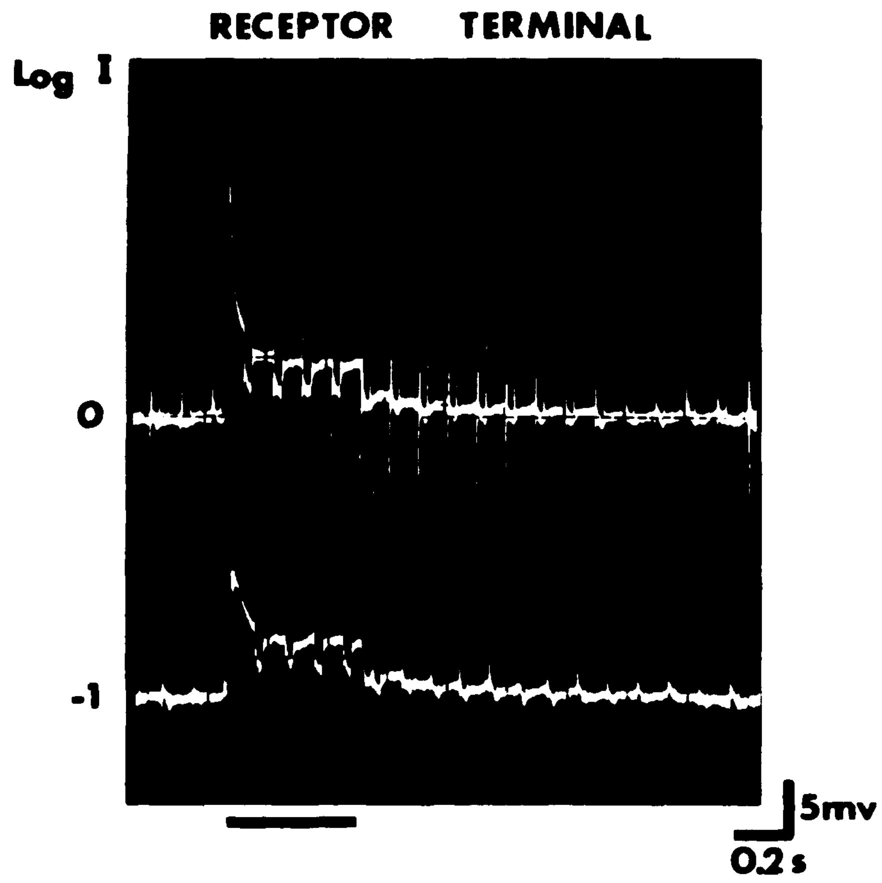


Figure 8

Figure 9

The OFF response in the receptor terminal was enhanced by increasing the duration and frequency of the stimulus. Upper: In the dark adapted preparations the OFF response following a 0.45 sec test (every 6 sec) flash was not very pronounced. Middle: When the duration and frequency of the stimulus was increased (0.9 sec duration every 3.3 sec) the OFF response increased in amplitude and duration. Lower: The two responses shown above are superimposed. Note the enhanced OFF response following the longer test flash, although the dark potential was not significantly changed. The slight reduction in the sustained portion of the light response during the longer stimulus is probably the result of light adaptation. The 0.45 sec response was recorded from a dark adapted preparation while the 0.9 sec response was recorded under conditions of continuous test flashing.

Stimulus intensity: Log I = -1. Resting potential: -53 mv.

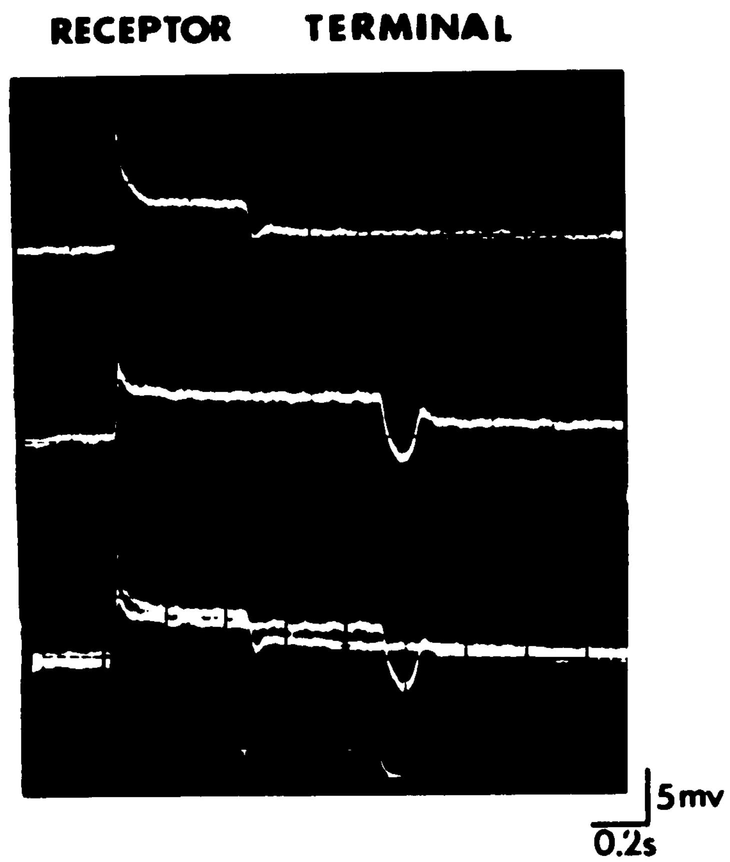


Figure 9

Figure 10

Increasing the duration and frequency of the test stimulus enhanced the OFF response in this receptor terminal at every light intensity above Log I = -4. The intensity response series shown on the left was recorded from a relatively dark adapted preparation. The longer responses were recorded from a relatively light adapted preparation after the stimulus conditions were changed. These responses were recorded from the same cell shown in figure 9. The data in this figure is represented in graph III.

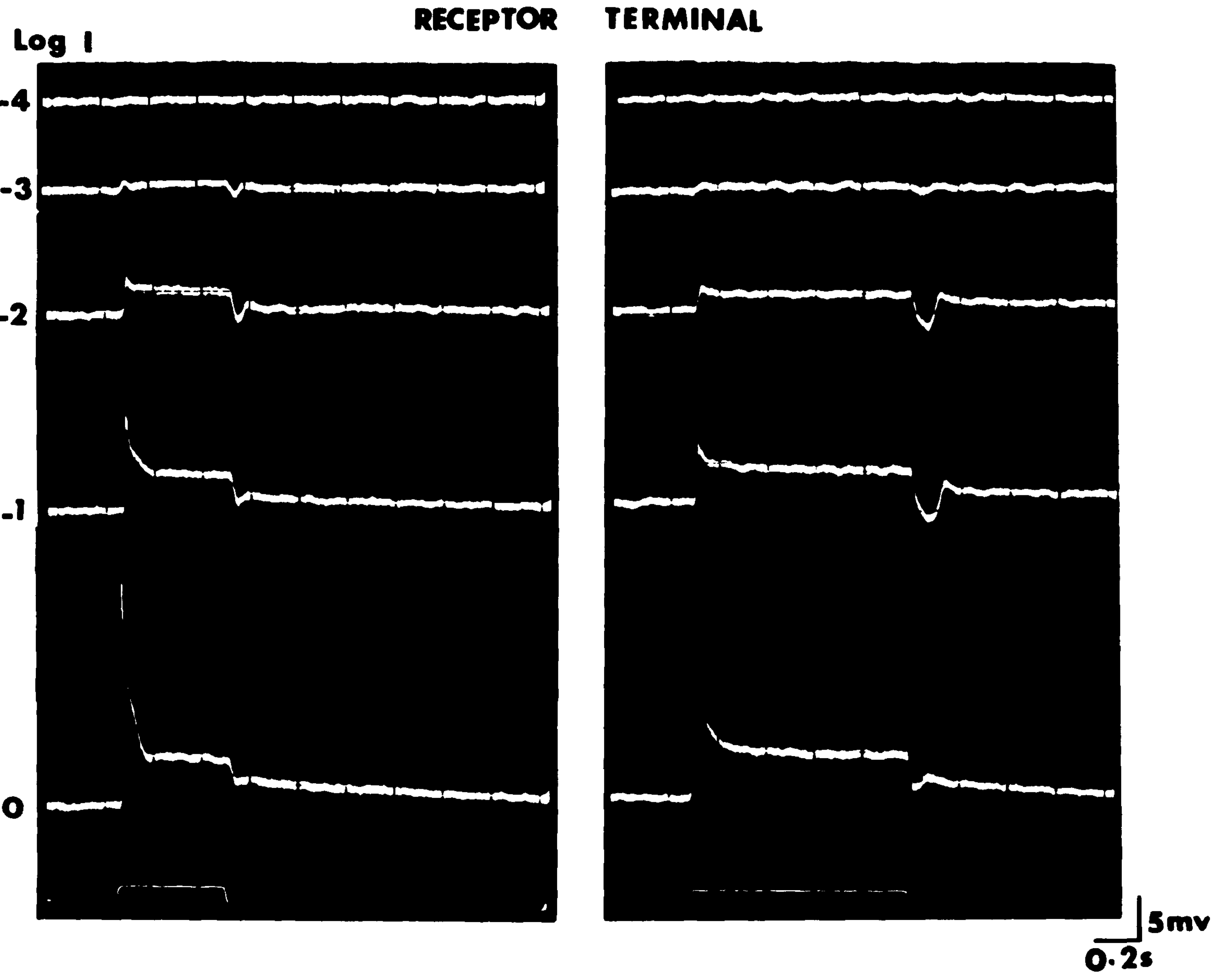


Figure 10

Figure 11

The receptor OFF response following a Log I = -1 test flash was enhanced by increasing the frequency and duration of the stimulus. This cell was impaled in the nuclear region of the receptor layer where the majority of photoreceptor responses were recorded. The records on the right (A,B,C) show 2-4 superimposed responses shown as part of a single sweep recorded at a slower sweep speed to the left. The response to a long duration (5.5 sec) flash (D, right) shows only 1 oscilloscope sweep (offset and onset of stimulus).
A: After 6 minutes of continuous test flashing (interval between flashes 6.3 sec) a typical OFF response was seen. As the interval between test flashes was decreased (B. 3 sec; C. 2.1 sec) while the stimulus duration was held constant, the OFF hyperpolarization was enhanced. When the duration of the stimulus was increased to 5.5 sec (D) the OFF response increased in amplitude and duration. E: Superimposed responses before (B) and after (D) the duration of the light stimulus was increased. The superimposed record in E (left) shows trace in B (left) and trace in D (left). The record on the right in E shows 4 superimposed oscilloscope sweeps showing the short duration response (1 sweep), the offset and onset of the long duration flash (1 sweep), the ON-transient and sustained portion of the long duration response (1 sweep), and the sustained portion of the long duration flash (1 sweep). These responses were recorded during the

Figure 11 (continued)

period shown in B and D. Note that the dark potential and the sustained portion of the light response did not vary significantly as the stimulus conditions were changed; only the OFF hyperpolarization was affected.

Resting potential: -48 mv.

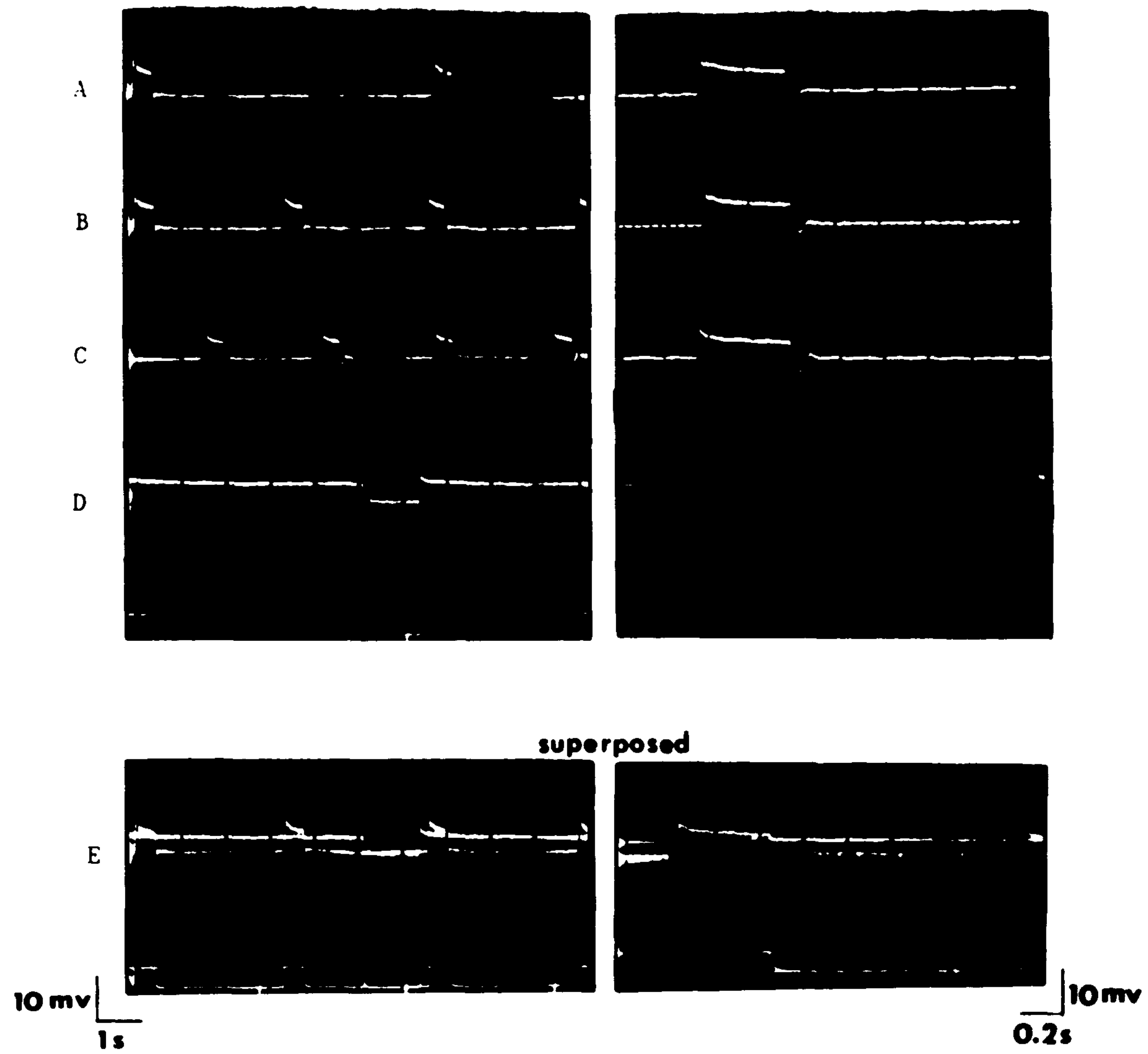


Figure 11

Figure 12

An OFF transient is normally not observed in response to an unattenuated (Log I = 0) test flash, but will appear if the duration of the stimulus is increased. These responses were recorded from the same cell shown in figure 11. In A, B, and C, the records on the left show 8 superimposed responses recorded as part of a single slow sweep shown to the right. The record in D (left) shows only 1 oscilloscope trace. A: Responses to Log I = 0 test flashes after the preparation had been dark adapted. B: Responses after 13 minutes of continuous test flashing under the same stimulus conditions. There was a small reduction in the size of the ON-transient, probably as a consequence of light adaptation. Note that the dark potential between test flashes was not altered. C: These responses were recorded 12 minutes after the frequency of the stimulus was increased. There was a dramatic reduction in the size of the ON transient, thus causing the ON-spike to appear more distinct. The interflash potential was essentially unchanged and a prominent OFF response was not seen. D: The response to the same unattenuated stimulus after the duration was increased from 0.4 sec to 5 sec. The dark potential just prior to light ON was 6 mv more negative than the dark adapted potential and an OFF oscillation appeared at the termination of the stimulus.
Resting potential (dark adapted): -48 mv.

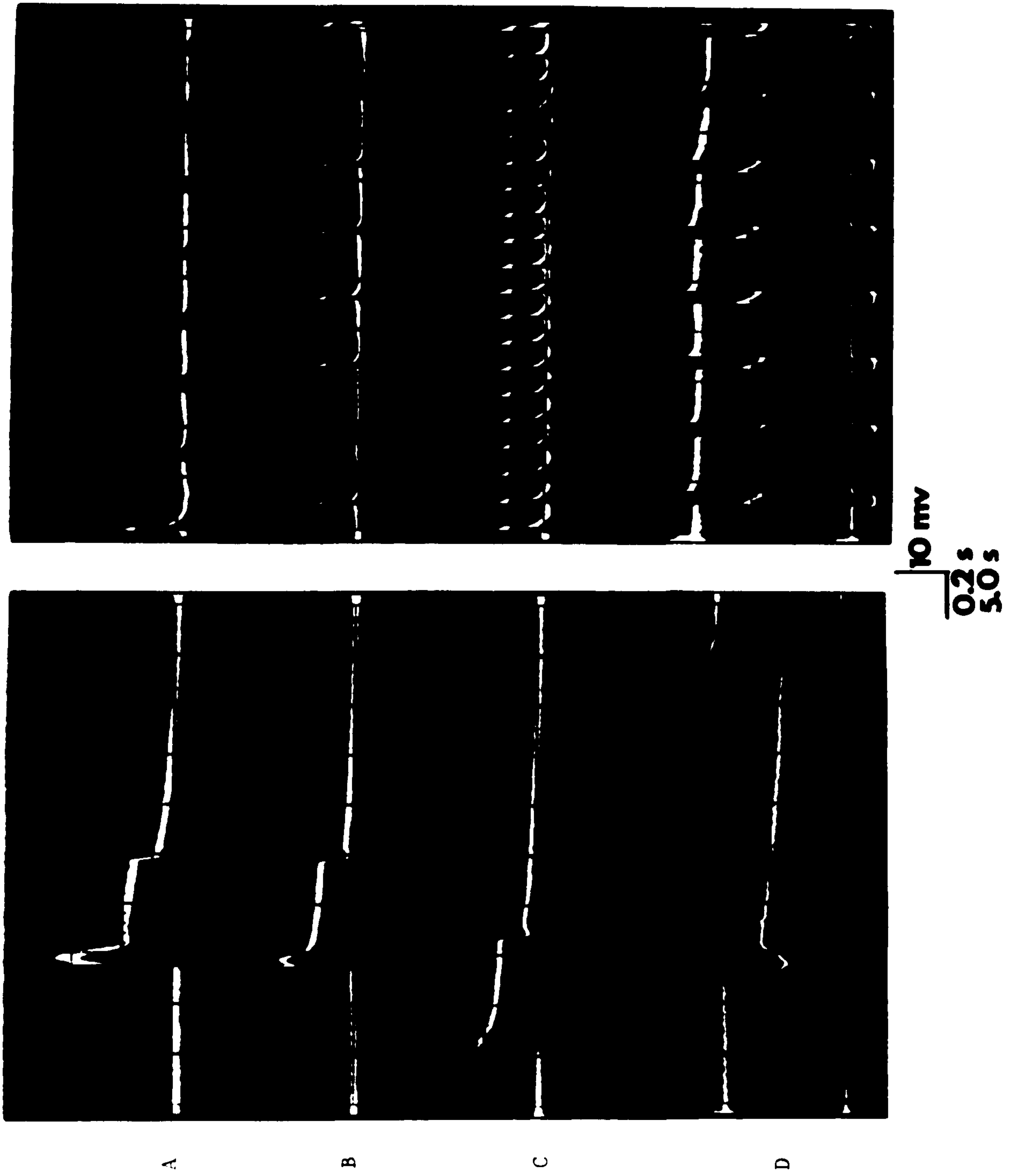


Figure 12

Figure 13

Superimposed responses selected from the records shown in the preceding figure (figure 12). These records were recorded at the fast sweep speed (0.2 sec per division). A: Superimposed oscilloscope sweeps showing one response from C in figure 12 and 1 response from D in figure 12, before and after the duration of the test flash was increased from 0.4 to 5 sec. Note that the ON-transient and sustained portions of the light responses in each record coincide, but the dark potential preceding the long duration (5 sec) stimulus is 6 mv more negative than the dark potential preceding the short (0.4 sec) stimulus. B: Four superimposed oscilloscope traces taken from C and D in figure 12, showing ON-transient, sustained, and OFF responses before and after the duration of the stimulus was increased. These records (B) were recorded at the fast sweep speed (0.2 sec per division). This record shows 2 short (0.4 sec) duration responses from figure 12, C; 2 long (5 sec) duration responses from figure 12, D (one showing ON-transient and sustained response as in the top record shown above, and one showing sustained and OFF response). Note that the ON-transient and sustained components of the 4 light responses are almost identical but the dark potential just before the 5 sec response has shifted about 6 mv more negative.

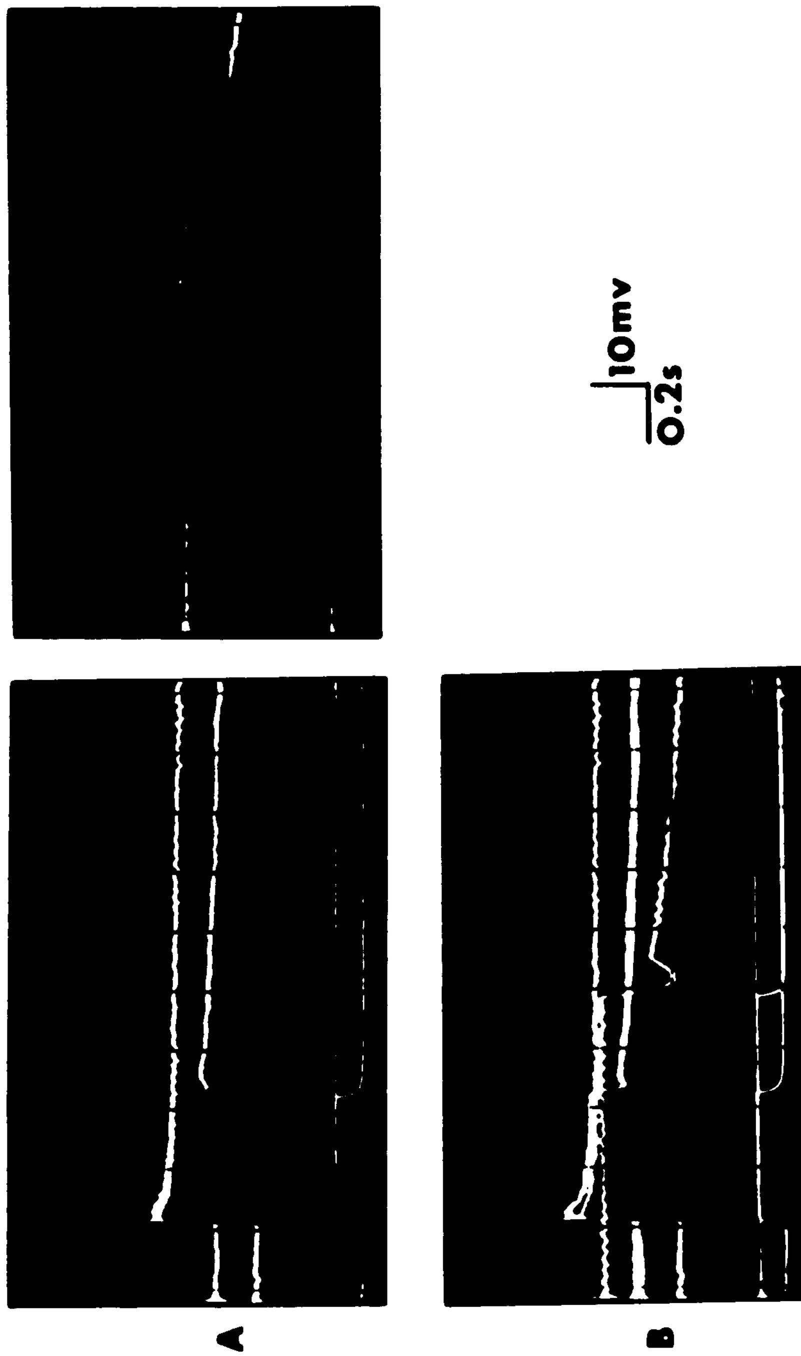


Figure 13

Figure 14

The OFF response in a photoreceptor from a different intact nerve preparation was enhanced by increasing the duration of an unattenuated test flash (Log I = 0). This preparation was washing in Ringer solution almost 4 hours before these records were taken. A dark adapted intensity response series from this cell is shown in figure 3. The OFF response following Log I = 0 test flashes is normally not very prominent. The upper records show the response to constant intensity (Log I = 0) flashes as the stimulus duration was increased from 0.4 to 3 sec. The responses shown at the top (0.4 sec duration) were recorded after 10 minutes of continuous test flashing of constant intensity, duration, and frequency. As the duration of the stimulus was increased a prominent hyperpolarizing OFF transient appeared. The dark potential between test flashes did not vary significantly although there was a slight negative shift in dark potential following the longest stimulus. The lower record shows 4 superimposed responses at each stimulus duration. Note the dramatic enhancement in the OFF response while the sustained portion of the light response was relatively unaffected. The data in this figure is represented in Graph IV.

Resting potential: -40 mv.

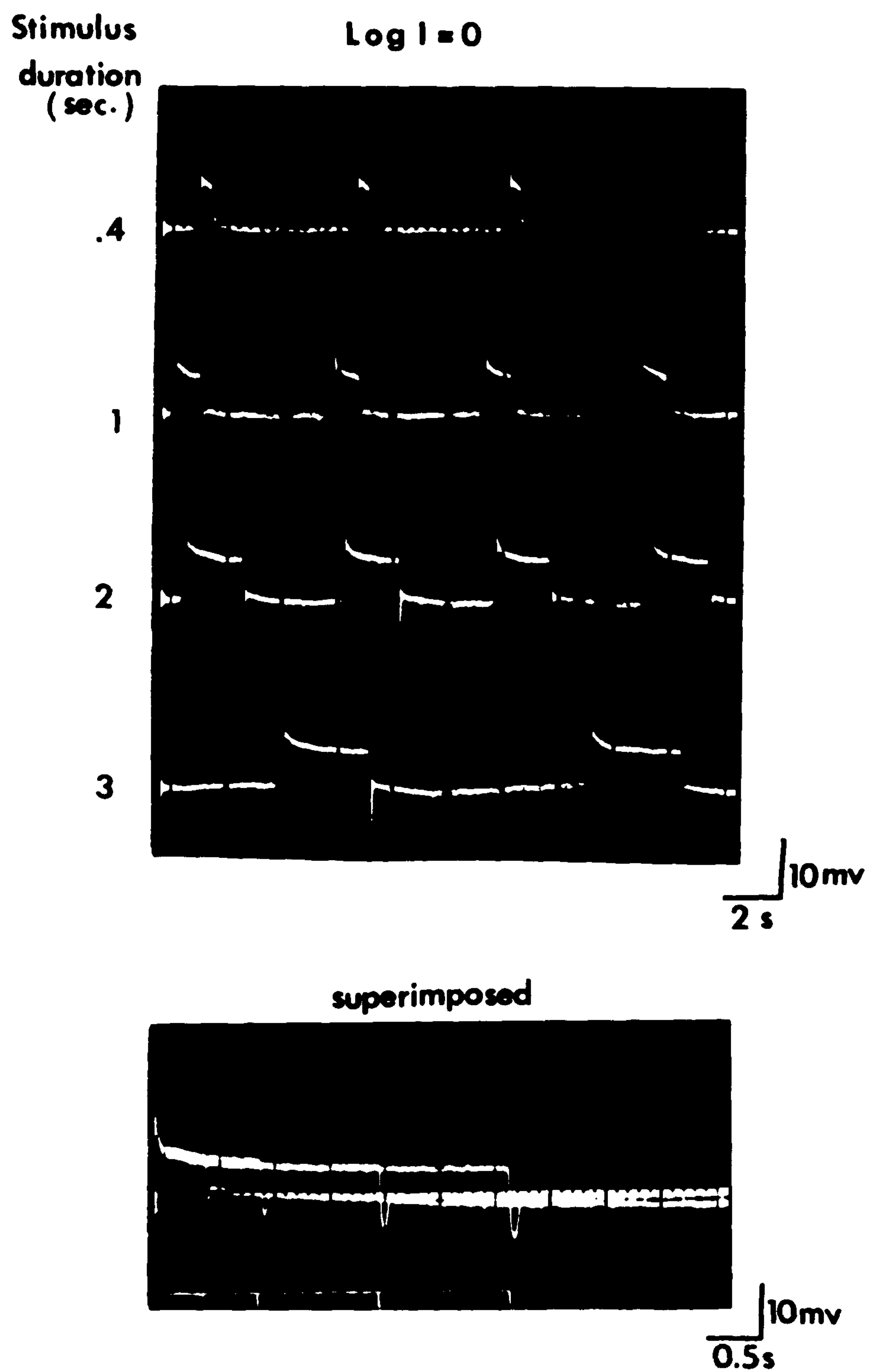


Figure 14

Figure 15

Intensity response series showing typical L-neuron responses from an intact nerve preparation. This cell displayed regular light inhibited oscillations following test flashes of moderate intensity. This type of dark oscillatory activity was considered to be within the normal range of L-neuron responses. The OFF response was more "oscillatory" in appearance than the cell shown in figure 16. Stimulus intensity increases from top to bottom.
Resting potential: -43 mv.

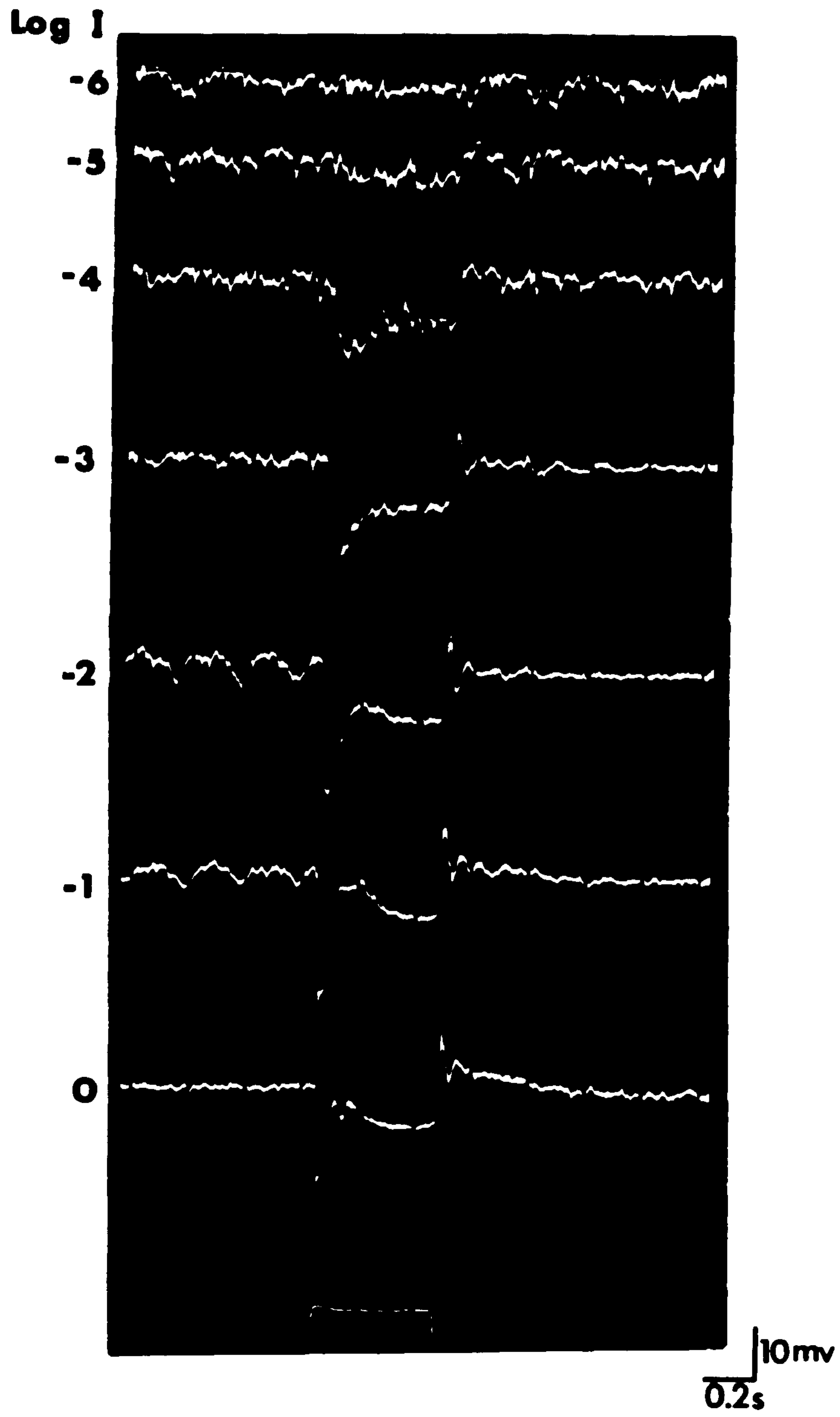


Figure 15

Figure 16

Intensity-response series showing typical L-neuron responses from an intact nerve preparation. This cell showed the usual noisy voltage fluctuations in the dark but there was no regular oscillatory activity. The OFF response recorded from this cell has a spike-like appearance. Stimulus intensity increases from top to bottom.

Resting potential: -47 mv.

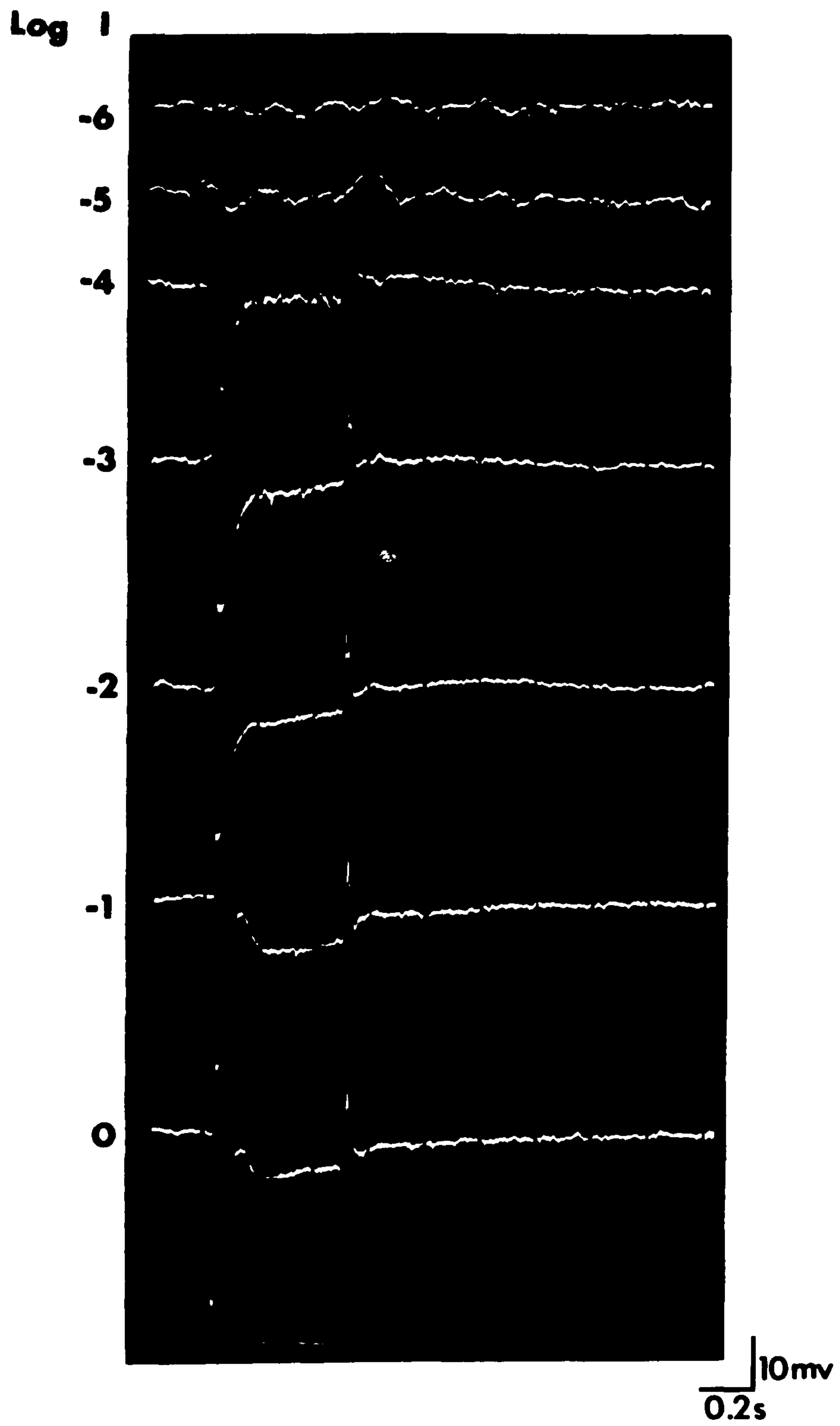


Figure 16

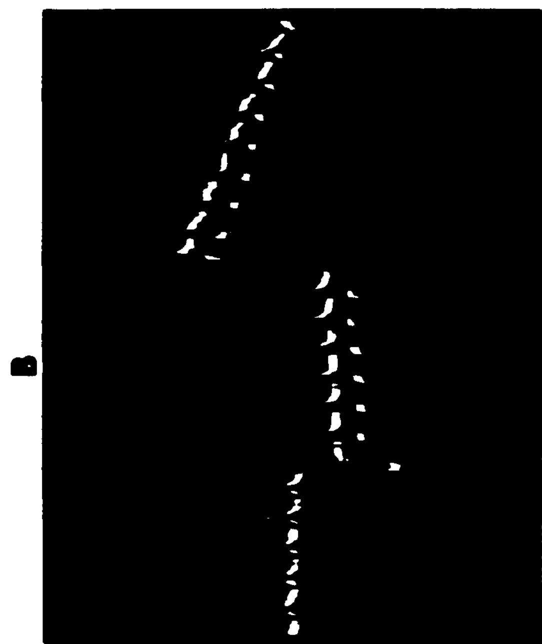
Figure 17

Resistance changes in L-neuron responses recorded from two different intact nerve preparations. The waveform of the L-neuron response in A was typical of the majority of L-neuron responses studied. The response in B showing a less phasic light response and a large slow OFF depolarization was observed less frequently, but both types of responses were occasionally recorded from the same preparation. Resistance changes were monitored by passing depolarizing pulses (1 nanoamp for the left, 0.6 nanoamp for the right) through a bridge circuit. A downward deflection of the pulse on portion of the trace represents a decrease in resistance. A 1 mv defection represents a resistance change of 1 megohm for the response shown in A and 3 megohms for the response shown in B.

The resistance decrease following the termination of the test flash was larger than that observed during illumination. The resistance decrease in the response shown in A persisted after the potential had recovered to the dark adapted level. The resistance decrease following the response shown in B persisted for the duration of the OFF depolarization. The resistance changes shown here were observed in more than 30 consecutive responses in each case.

Stimulus intensity: Log I = -2 (A); Log I = -1 (B).

Resting potential: -56 mv (A); -58 mv (B).



5mv
0.2s

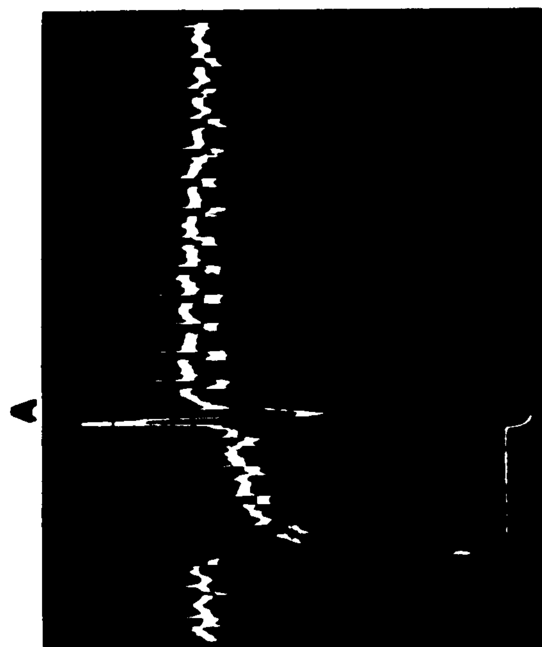
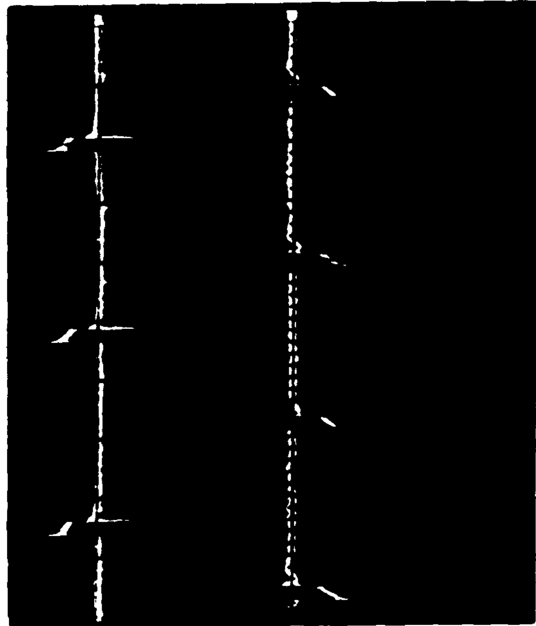


Figure 17

Figure 18

Typical intracellular responses from photoreceptor and L-neuron recorded under constant stimulus conditions. An intensity response series from each cell is shown in figures 3 and 16. The records on the left show superimposed responses to consecutive test flashes shown as part of a single sweep recorded at the slower sweep speed to the right. These cells did not show any spontaneous oscillatory activity in the dark. If stimulus conditions remained constant, consistently stable responses like these could be recorded for 4 or more hours. These cells were impaled in two different intact nerve preparations.
Stimulus intensity: Log I = -2.



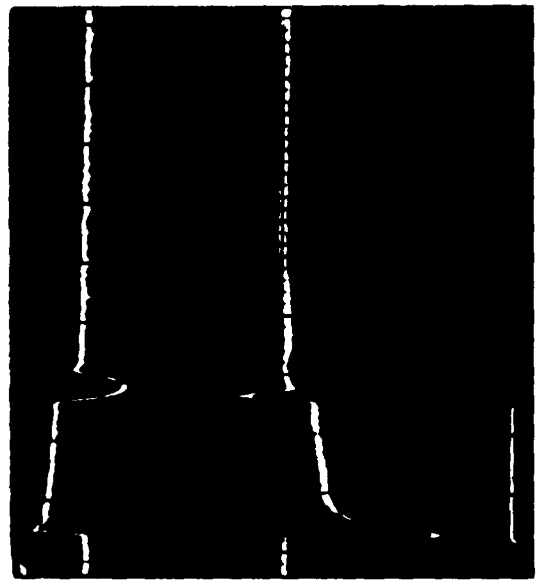


Figure 18

Figure 19

Typical intracellular responses from photoreceptor and L-neuron recorded from two different intact nerve preparations under constant stimulus conditions. An intensity-response series from each cell is shown in figures 2 and 15. The records on the left show superimposed responses to consecutive test flashes shown as part of a single sweep recorded at the slower sweep speed to the right. These cells showed small light-inhibited oscillations following test flashes of critical intensity. Under the stimulus conditions shown here they appeared approximately 1.5 seconds following light OFF. As long as the stimulus conditions remained constant, the waveform of the light responses and the pattern of the dark oscillations did not vary. Oscillatory activity such as that shown here was seen in approximately 15% of the cells examined.

Stimulus intensity: photoreceptor, Log I = -1; L-neuron, Log I = -2.

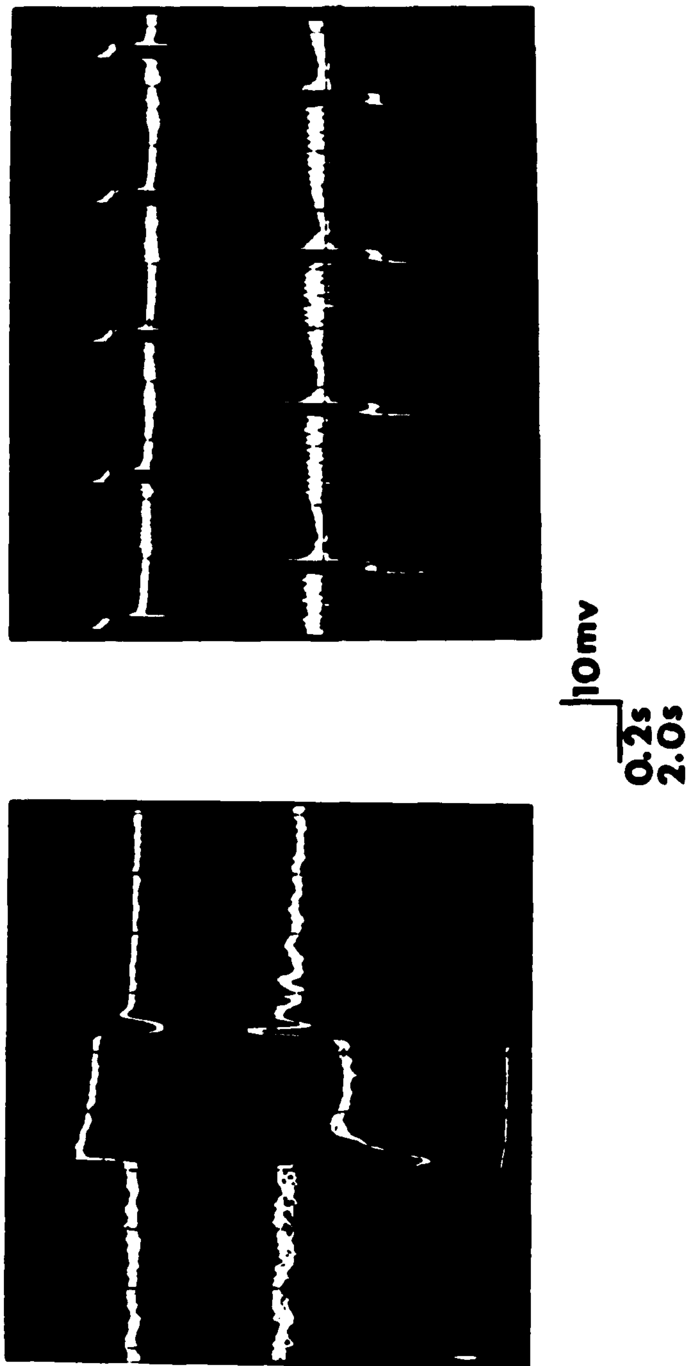


Figure 19

Figure 20

Intensity-response series showing light-excited spiking unit impaled in the synaptic region of the ocellus. This cell was impaled when the preparation had been washing 5 minutes following the application of 0.14 mM curare for 45 minutes. When the cell was first penetrated, spontaneous dark impulse activity (in addition to the light-evoked spikes) was observed, but the dark impulses subsided in less than one minute. The firing pattern varied with stimulus intensity in a complex manner. In general, the spike frequency was highest at the beginning of each test flash. No spikes were observed in response to the dimmest test flashes (Log I = -4; I = -5).

Resting potential: -46 mv.

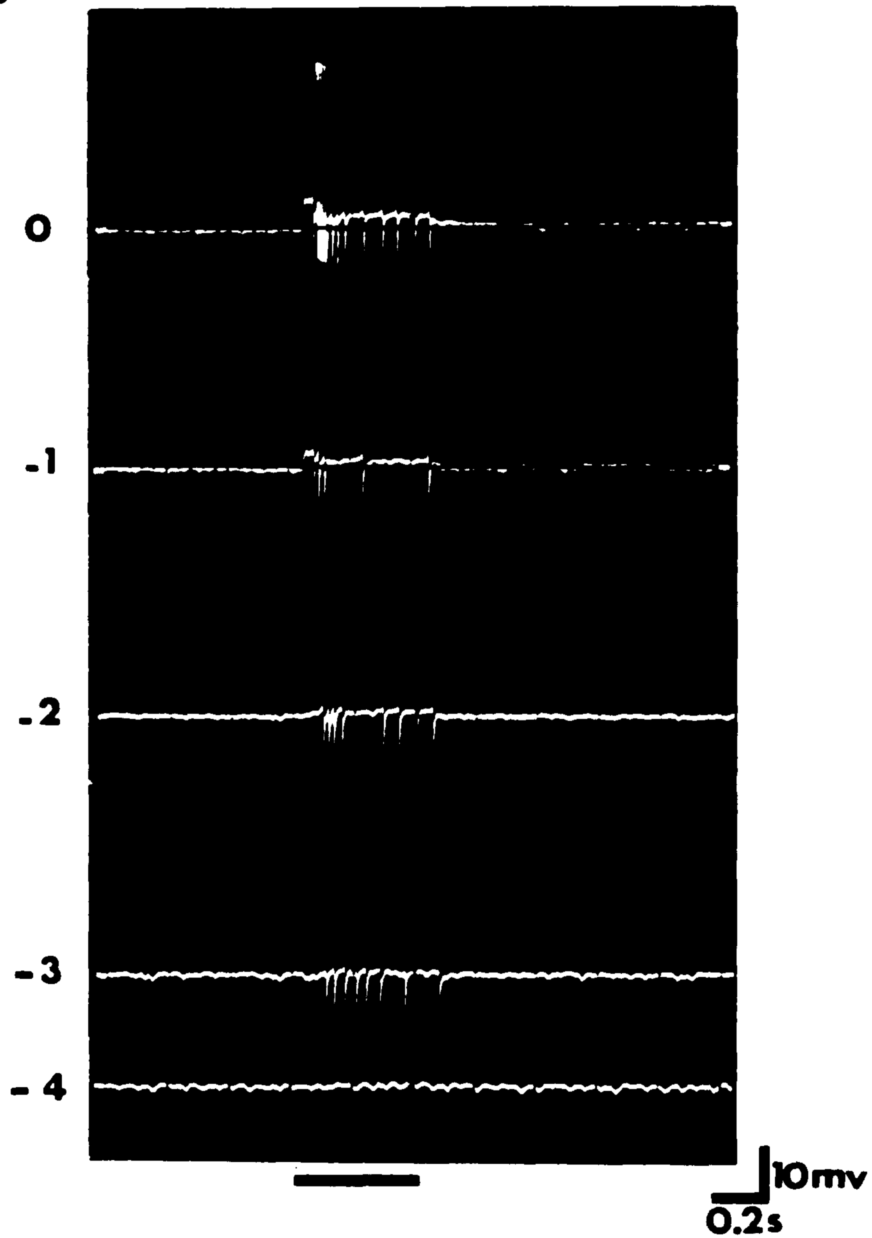


Figure 20

Figure 21

Intensity-response series from a unit showing impulse activity following the termination of the light stimulus. This process was impaled in the synaptic region of the ocellus and is very similar to a spiking unit described in earlier reports (Dowling and Chappell, 1972; Klingman, 1976) except that in the cell shown here the impulses did not persist in the dark. At higher light intensities the first spike is larger than the others. Note the large sustained OFF response.

Resting potential: -63 mv.

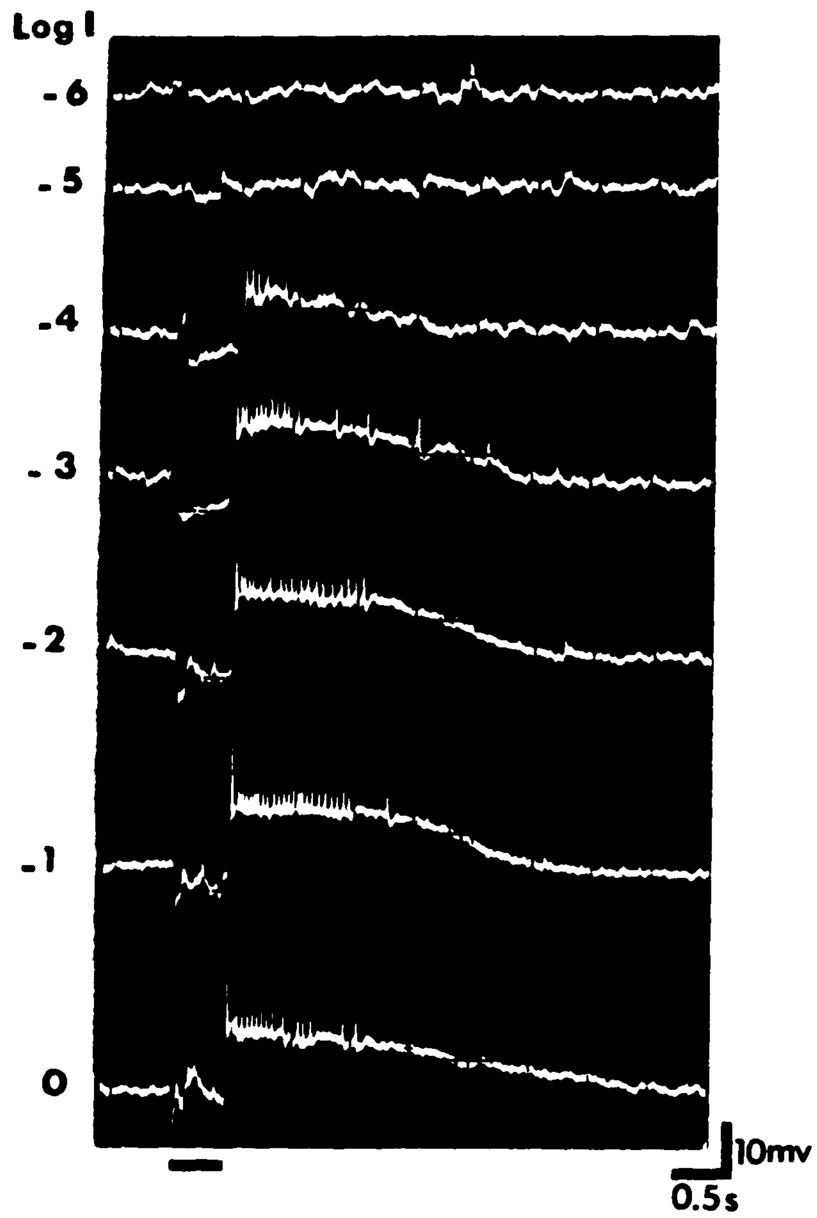


Figure 21

Figure 22

Photoreceptor and L-neuron responses recorded from two different cut nerve preparations under constant stimulus conditions. The records on the left show superimposed responses to 4 consecutive test flashes shown as part of a single sweep recorded at the slower sweep speed to the right. Severing the ocellar nerve did not eliminate the OFF response in the receptor and L-neuron. Both of these cells showed spontaneous oscillatory activity in the dark that was inhibited under these stimulus conditions. Note that the early "spike-like" character of the L-neuron OFF response was not noticeably different than in intact nerve preparations.

Stimulus intensity: Log I = -2.

Resting potential: photoreceptor, -40 mv; L-neuron, -50 mv.

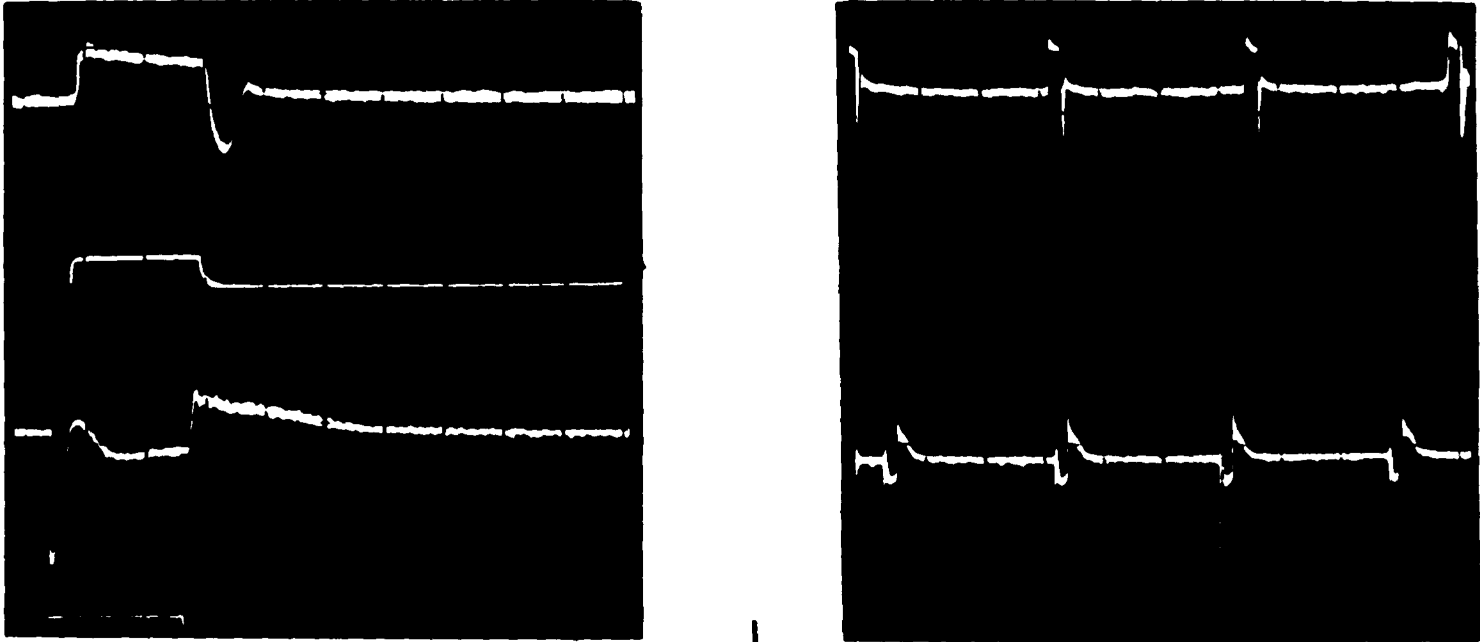


Figure 22

Figure 23

Intensity-response series showing L-neuron responses from a cut nerve preparation. The "noisy" discrete hyperpolarizations in the dark are quite prominent. The OFF response is larger, more prolonged, and more oscillatory when compared to intact nerve preparations although the light response during illumination is not unusual. The OFF response lost its oscillatory appearance during stimulus conditions of continuous test flashing (see figure 44). Stimulus intensity increases from top to bottom. Resting potential (dark potential just prior to the test flash): -52 mv.

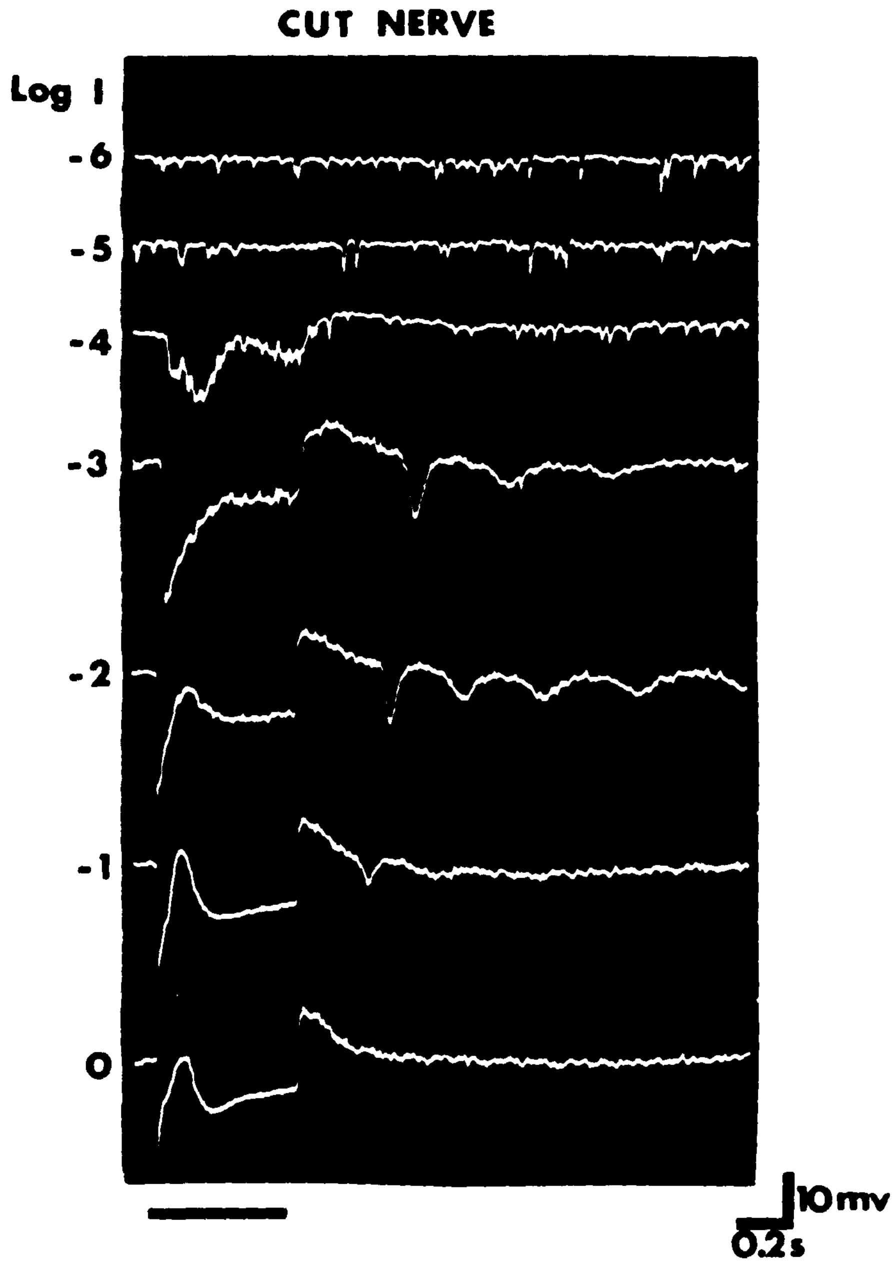


Figure 23

Figure 24

Intensity-response series showing L-neuron responses recorded from a different cut nerve preparation than that shown in figure 23. Note the large sustained OFF response showing "noisy" voltage fluctuations during the sustained depolarization. The early phase of the OFF response following more intense stimuli had a spike-like appearance. Stimulus intensity increases from top to bottom. Resting potential: -60 mv.

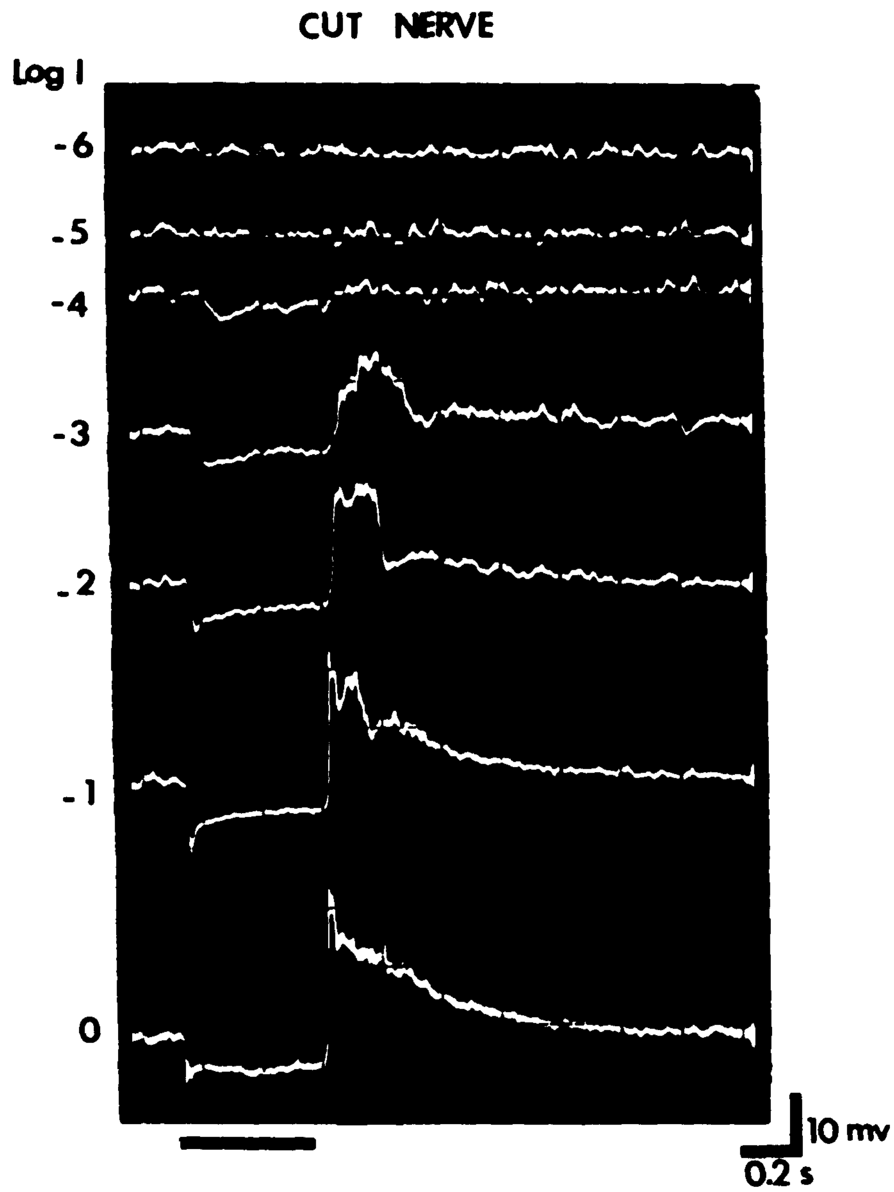


Figure 24

Figure 25

Resistance changes recorded from an L-neuron in a cut nerve preparation. Resistance changes were monitored by passing 1 nanoamp depolarizing pulses through a bridge circuit. A downward displacement of the pulse-ON portion of the trace represents a resistance decrease, with a 1 mv deflection approximately equivalent to 1 megohm. A large decrease in resistance (1-4 megohm) persisted for the duration of the OFF depolarization, and was seen in 12 consecutive responses.

Stimulus intensity: Log I = -2.

Resting potential: -50 mv.

CUT NERVE

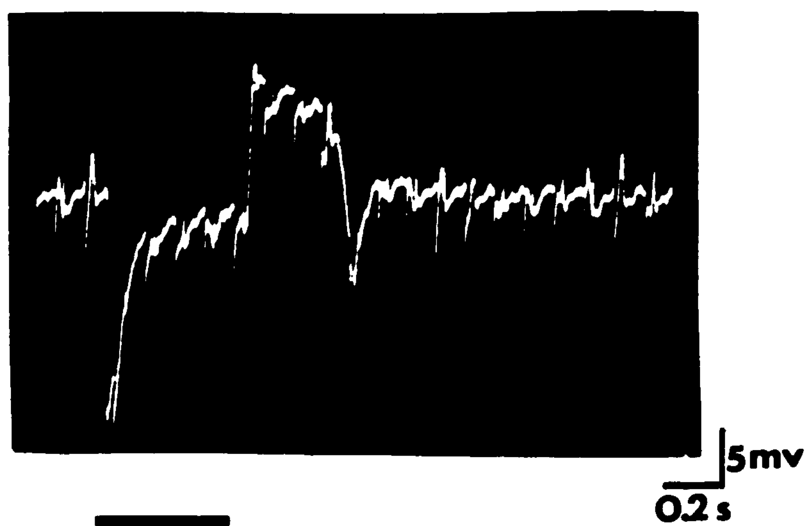


Figure 25

Figure 26

Intensity-response series showing photoreceptor responses from cut nerve preparation. Note the prominent OFF response and large sustained hyperpolarization following the brightest test flash (Log I = 0). An OFF response was observed following the dimmest (Log I = -5) test flash although a light-evoked depolarization was not detectable at this stimulus intensity.
Stimulus intensity increases from top to bottom.
Resting potential (dark adapted): -48 mv.

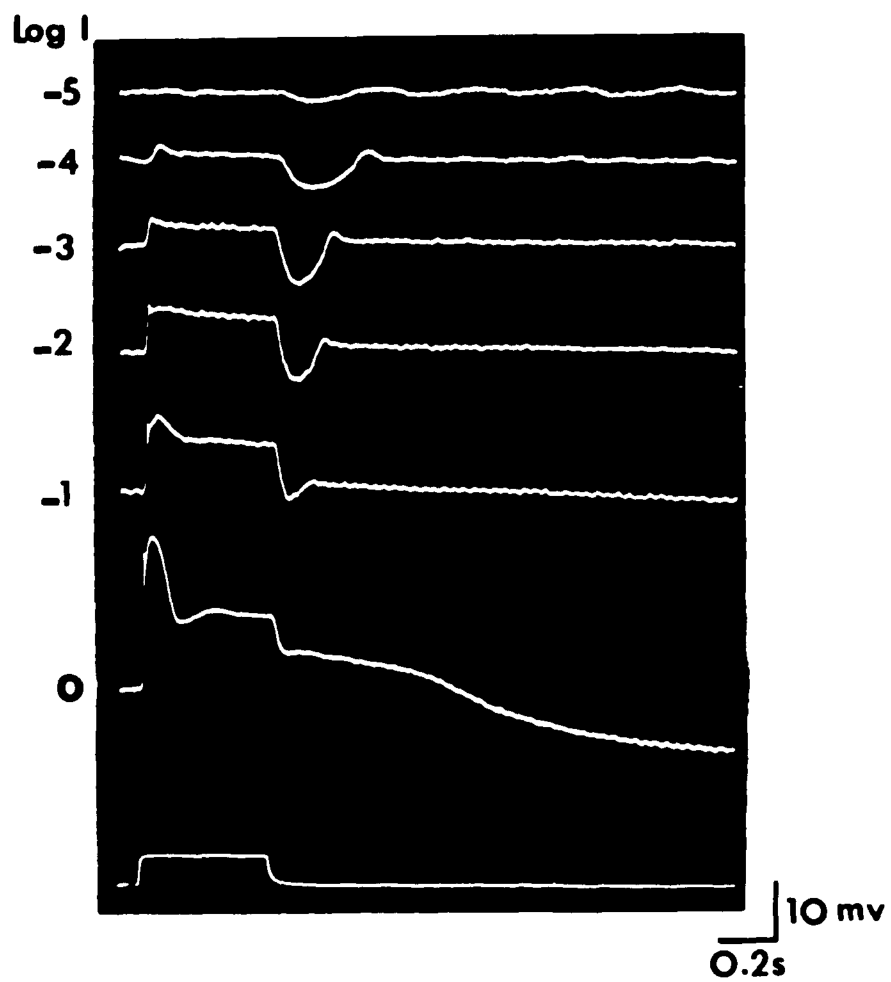


Figure 26

Figure 27

Intensity-response series showing photoreceptor responses from a different cut nerve preparation than in figure 26. This cell displayed continuous oscillatory activity in the dark. Note prominent OFF response following dim (Log I = -5) stimulus and delayed sustained hyperpolarization following brightest (Log I = 0) stimulus. Stimulus intensity increases from top to bottom.
Resting potential: -50 mv.

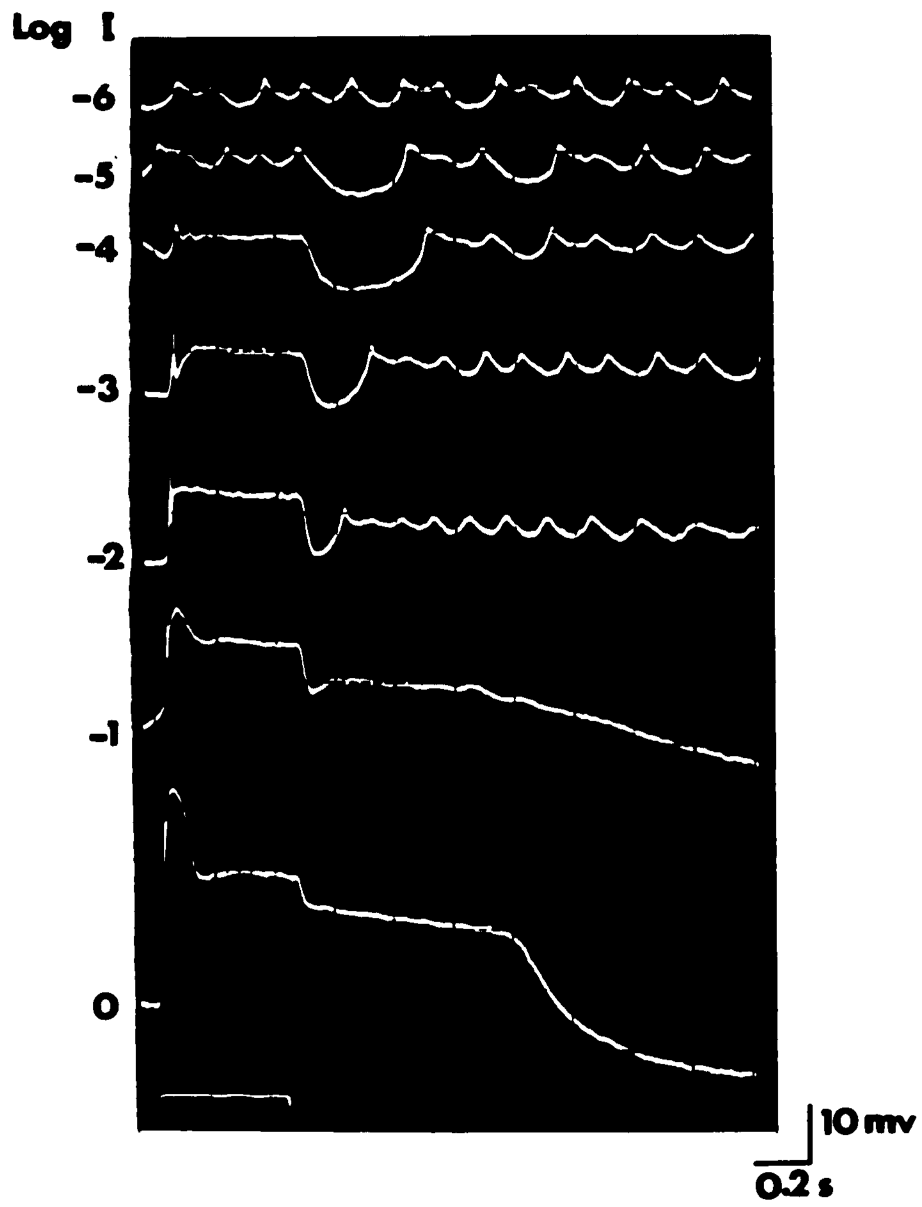


Figure 27

Figure 28

Intensity-response series showing responses from presumed receptor terminal in cut nerve preparation. Note prominent OFF response, even though the ocellar nerve had been isolated from the brain. This cell was impaled in the synaptic region of the ocellus and was lost shortly after these records were taken. Stimulus intensity increases from top to bottom.
Resting potential: -50 mv.

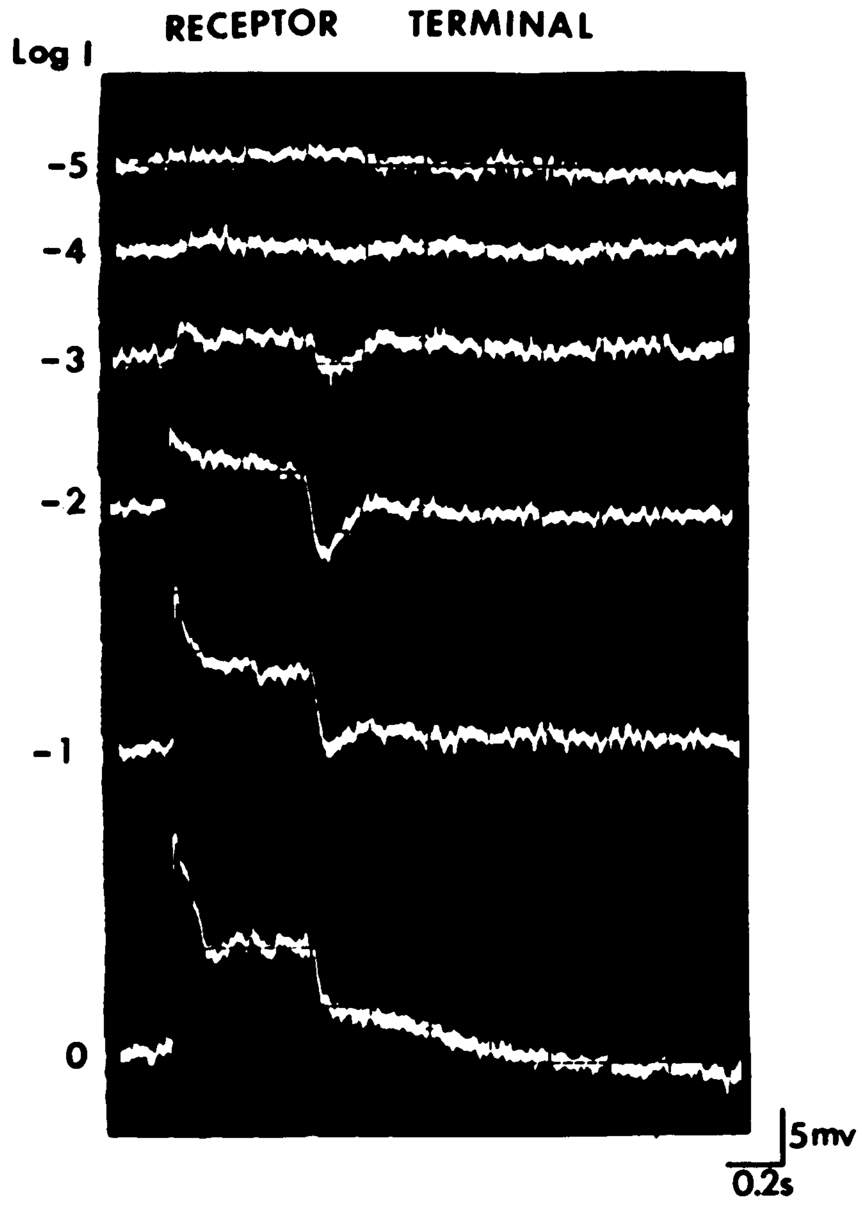


Figure 28

Figure 29

Intracellular recordings from a photoreceptor during electrical stimulation of the ocellar nerve. The application of a brief hyperpolarizing pulse (4 msec, 4 volt, brain electrode negative) to the ocellar nerve evoked a typical "OFF" type transient in the dark adapted photoreceptor. The latency from the onset of the stimulus to the onset of the evoked hyperpolarization was 14 msec. The stimulus was ineffective during light flashes and for up to 800 msec following light OFF. The latter period approximately corresponds to the period during which the conductance of the second order neuron remains increased (see figure 31). The stimulus artifact is the large deflection preceding the hyperpolarizing response.

Stimulus intensity: Log I = -2.

Resting potential: -47 mv.

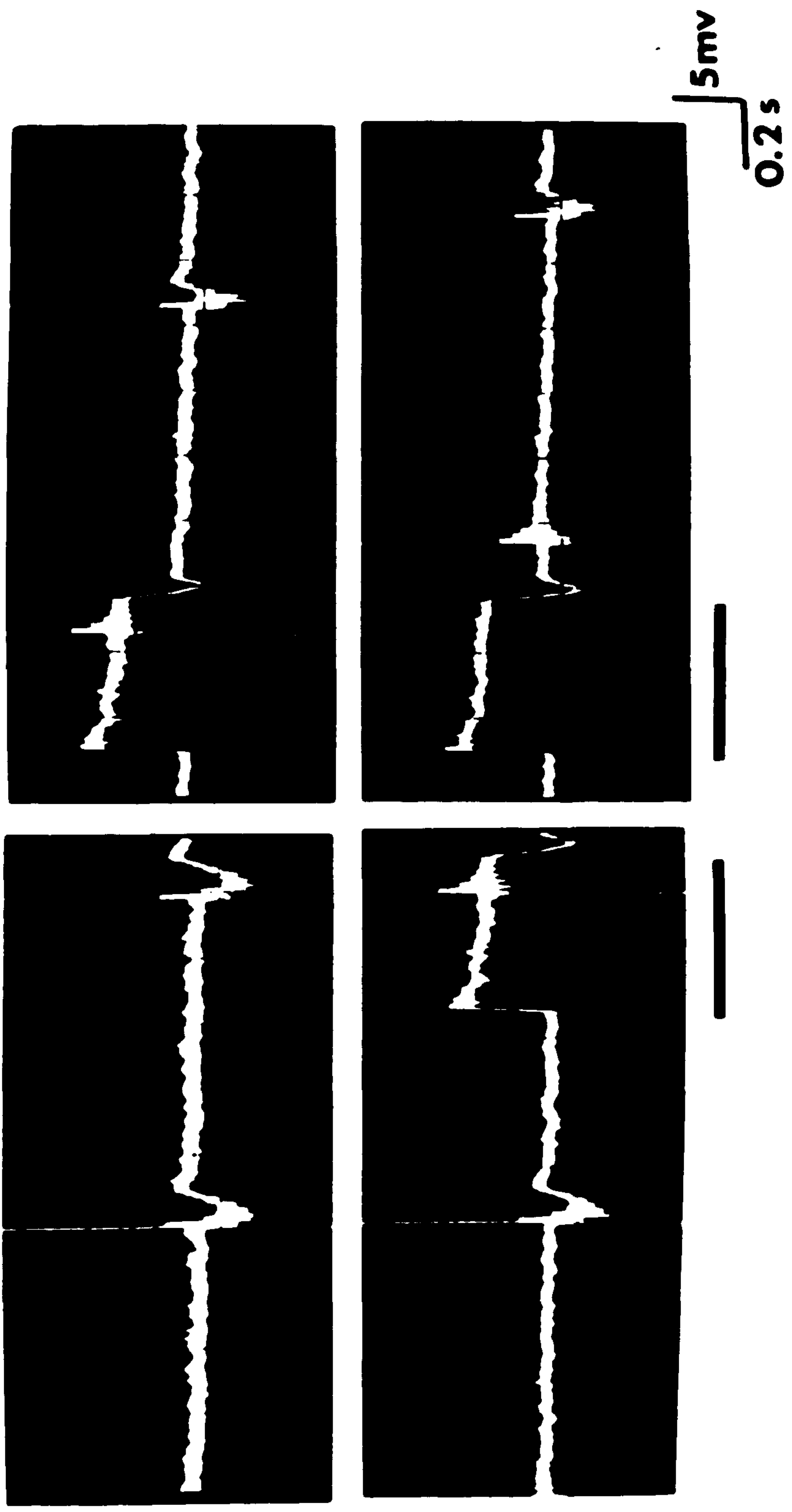


Figure 29

Figure 30

Intracellular recording from photoreceptor during antidromic stimulation of the ocellar nerve. This is the same experiment shown in the preceding figure (figure 29), but these records were taken at a slower sweep speed (0.5 sec/div). In the dark adapted condition (A) an OFF-type hyperpolarization followed every electrical stimulus. The stimulus was ineffective in evoking a response during light flashes (B) and for up to 800 msec following light OFF (C). The first detectable current evoked response following light OFF was less prominent than subsequent responses elicited 2 seconds or more after the test flash (C).
Stimulus intensity: Log I = -1.

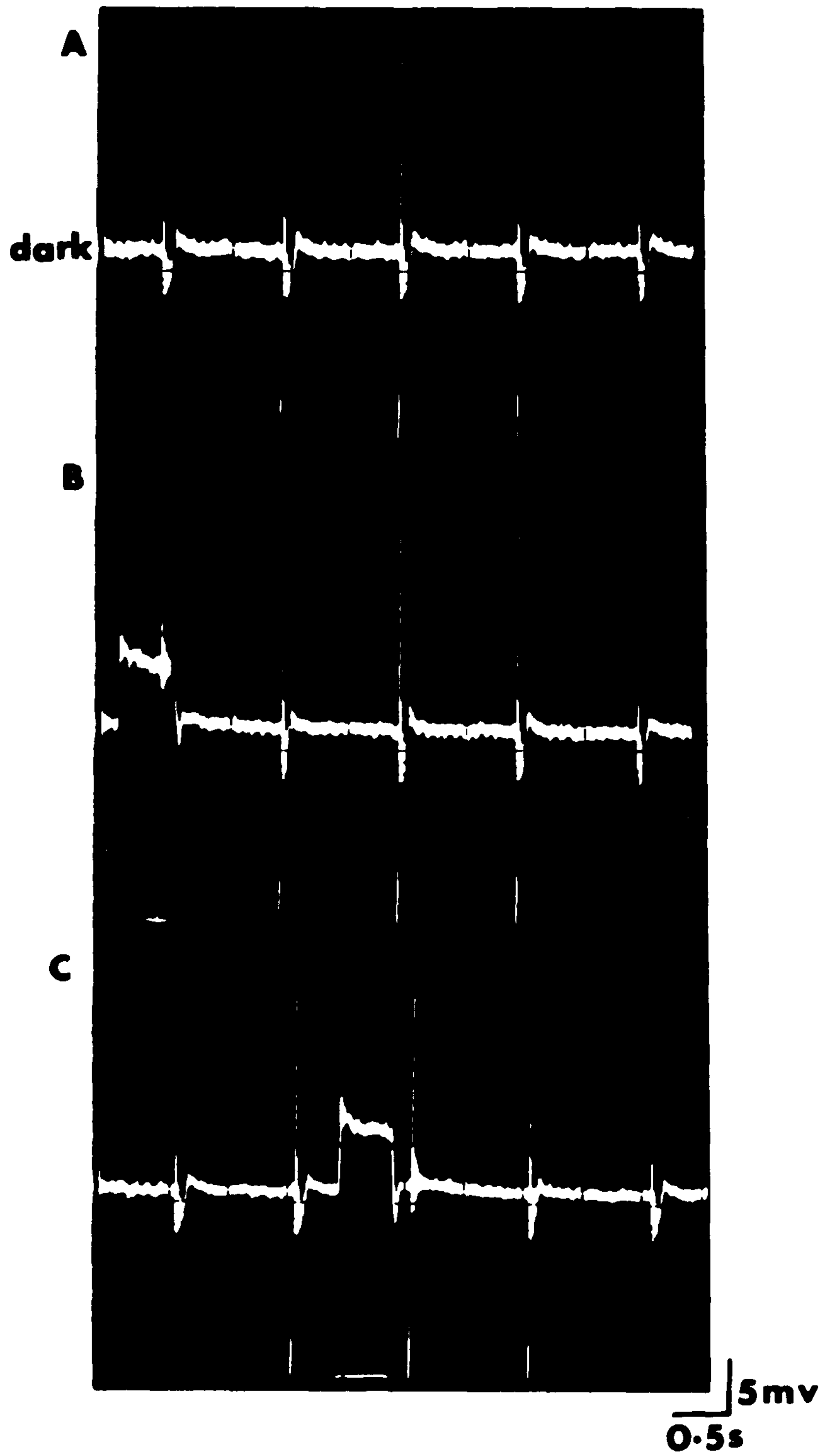
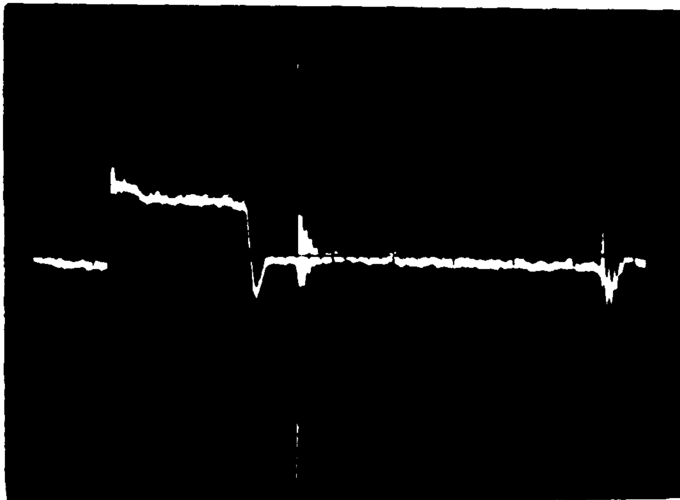


Figure 30

Figure 31

This figure compares the time course of the OFF conductance increase in the L-neuron with the dark period following light OFF when the antidromic stimulus was ineffective in eliciting an "OFF" response in the photoreceptor. These responses were recorded from the experiments shown in figures 17 and 29. The inability of the antidromic stimulus to evoke a response in the photoreceptor following light OFF period may be correlated with the prolonged conductance increase in the L-neuron.



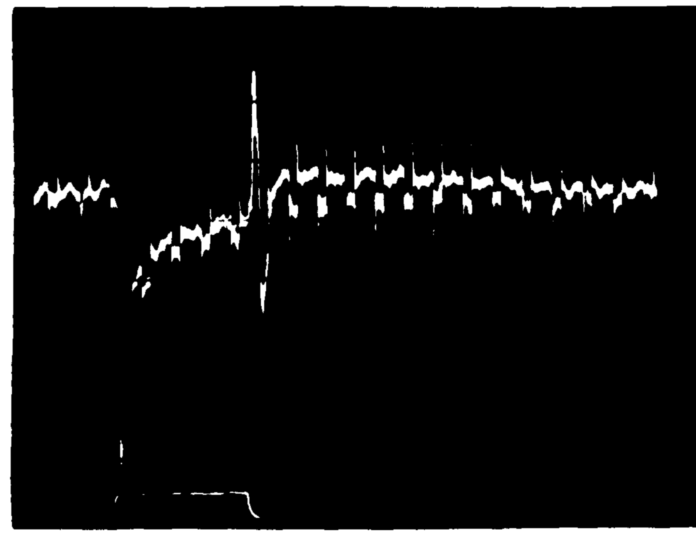


Figure 31

Figure 32

Photoreceptor and L-neuron responses recorded from two different cut nerve preparations under constant stimulus conditions. Each record on the left shows 4 superimposed responses to consecutive test flashes shown as part of a single sweep recorded at a slower sweep speed to the right. Both cells showed enhanced spontaneous oscillatory activity between test flashes. The dark potential in the photoreceptor seemed to fluctuate between the positive level reached following recovery from the OFF response (which would normally correspond to the dark adapted "resting" potential) and the negative value reached during the hyperpolarizing OFF response. Note the invariance of the depolarizing light response. The oscillations in the L-neuron consisted of transient paired hyperpolarizations followed by small positive overshoots. The shape of the oscillations in both cells were similar in appearance to normal light responses.

Stimulus intensity: photoreceptor, Log I = -2; L-neuron, Log I = -3.

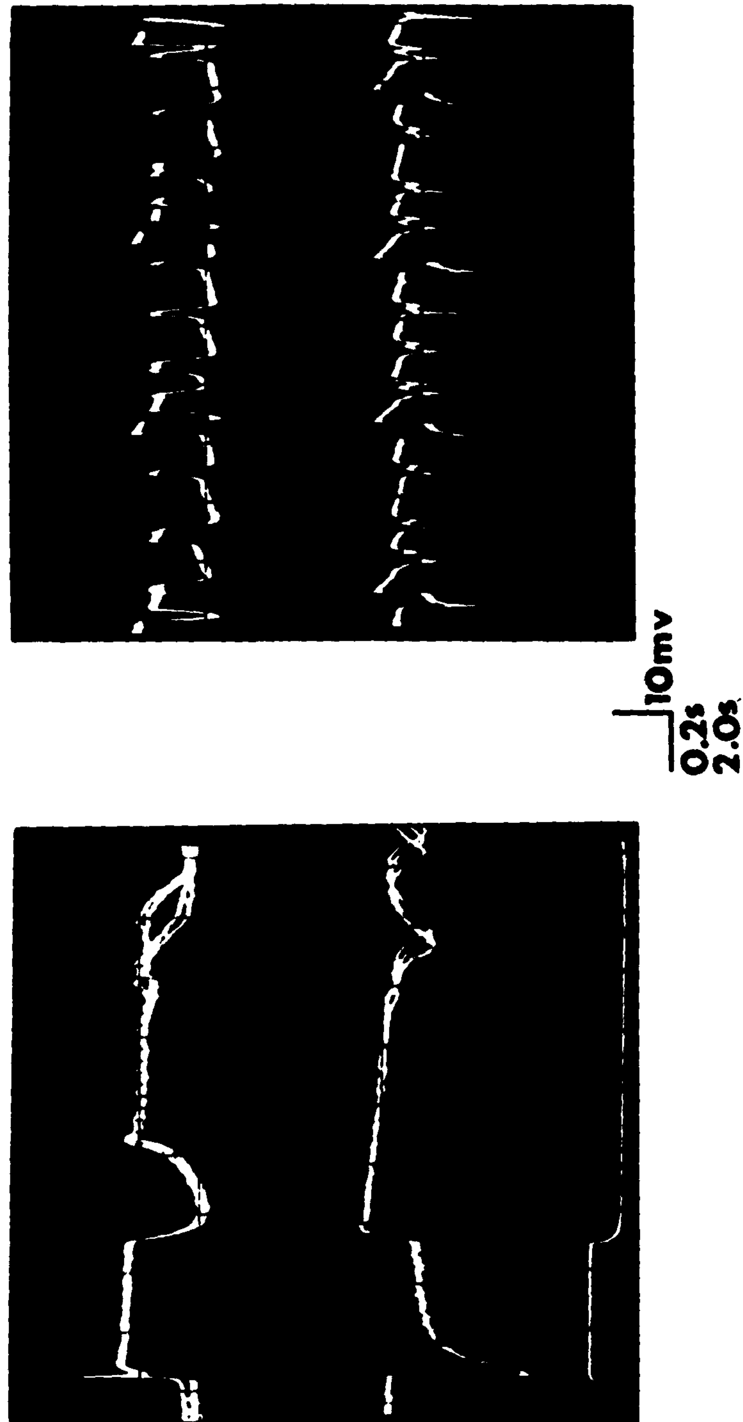


Figure 32

Figure 33

Intensity-response series showing L-neuron responses from a cut nerve preparation. This cell displayed pronounced oscillatory activity in the dark. The records on the left show superimposed responses to 4 consecutive test flashes shown as part of a single sweep recorded at the slower sweep speed to the right. The spontaneous hyperpolarizations were relatively unaffected by dimmer test flashes but were gradually inhibited as the stimulus intensity increased. At low light intensities the spontaneous hyperpolarizations were similar in appearance to the normal light response except they lacked a sustained component. The waveform and pattern of the dark oscillations seems to depend upon stimulus intensity. The oscillatory activity after 4 minutes in the dark was similar to that shown for the Log I = -6 record (see also figure 39). Note that the waveform of light response is fairly constant but the OFF response is more variable.

Resting potential: -50 mv.

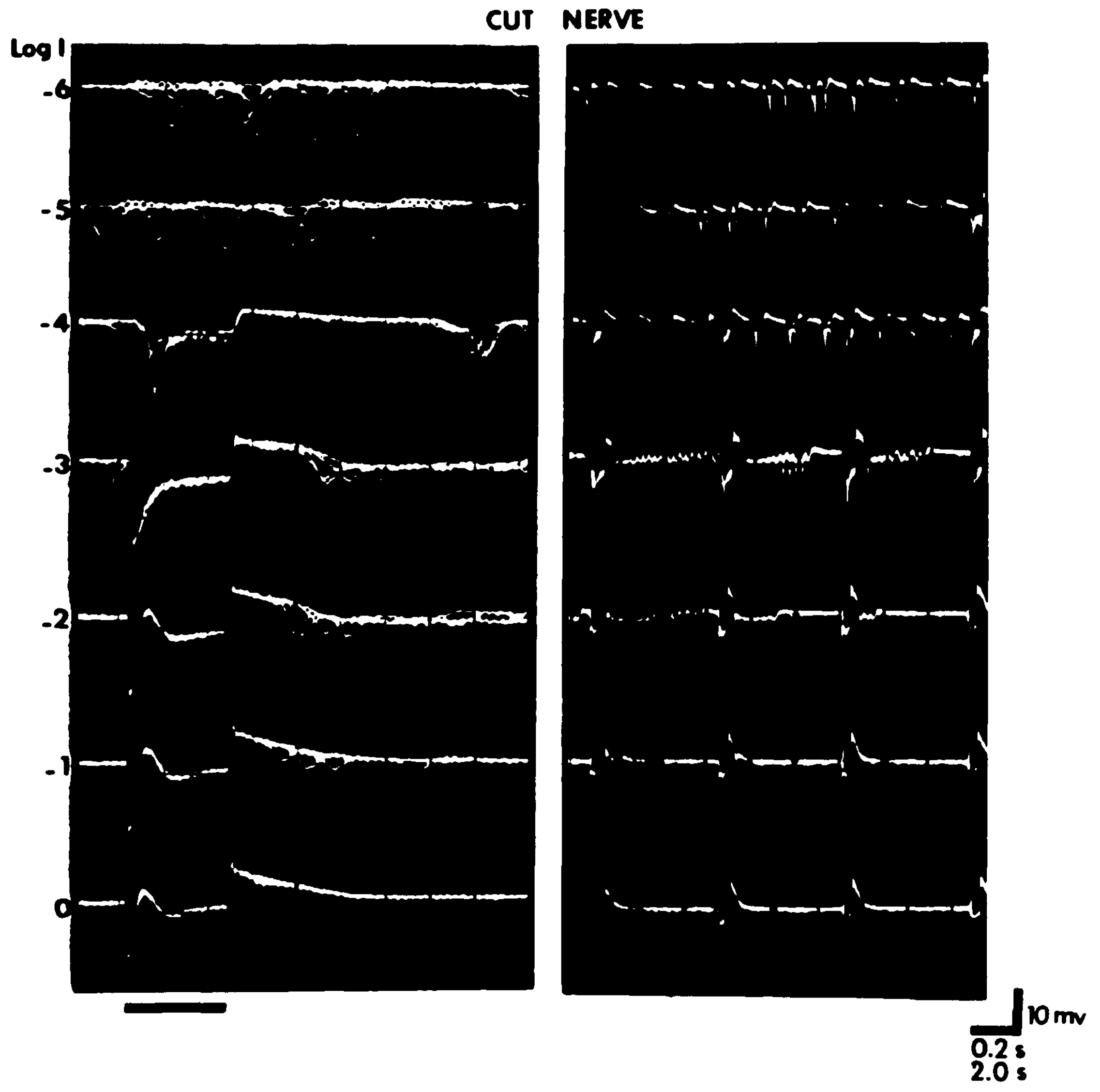


Figure 33

Figure 34

Intensity-response series showing photoreceptor responses from cut nerve preparation. This preparation exhibited unusually enhanced oscillatory behavior in the dark. The records on the left show superimposed responses to 4 consecutive test flashes shown as part of a single sweep recorded at a slower sweep speed to the right. The early portion of the first light response is not visible on the slow (2 sec per division) records. Note the invariance of the light-evoked depolarization, despite the fact that the dark potential just before light ON varied by more than 15 mv. The waveform of the oscillations resembled the normal light response, and were relatively unaffected by dimmer test flashes. In this preparation, bright test flashes did not completely inhibit the oscillations, but similar (though less pronounced) oscillatory activity was inhibited by bright flashes in most experiments. The presence of the ON-spike seems to depend upon the dark potential at light-ON.

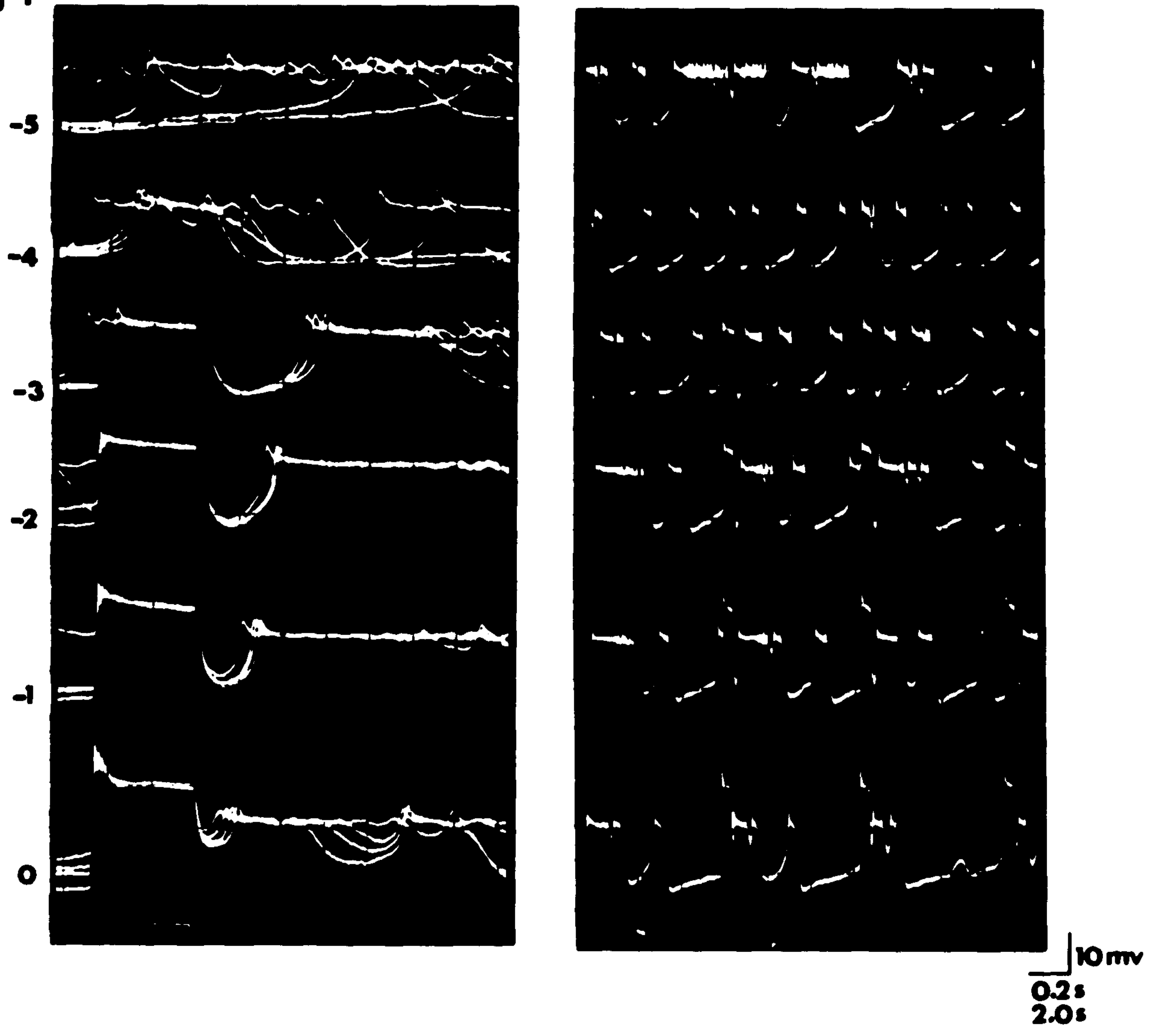


Figure 34

Figure 35

Post-illumination hyperpolarization following unattenuated light stimulus (Log I = 0). This cut nerve photoreceptor did not display very pronounced oscillatory behavior in the dark. The first test response to the dark adapted preparation showed a larger ON-transient than subsequent responses. The responses shown in B are superimposed on the slow sweep in C.

A: The preparation was placed in the dark following continuous I = 0 test flashing. The dark potential recovered in about 15 sec. (A').

B: Continuous I = 0 test flashing was initiated after 2 minutes in the dark.

C: The responses shown in B plus 1 sweep in the dark are superimposed.

This is the same cell as shown in figure 26.

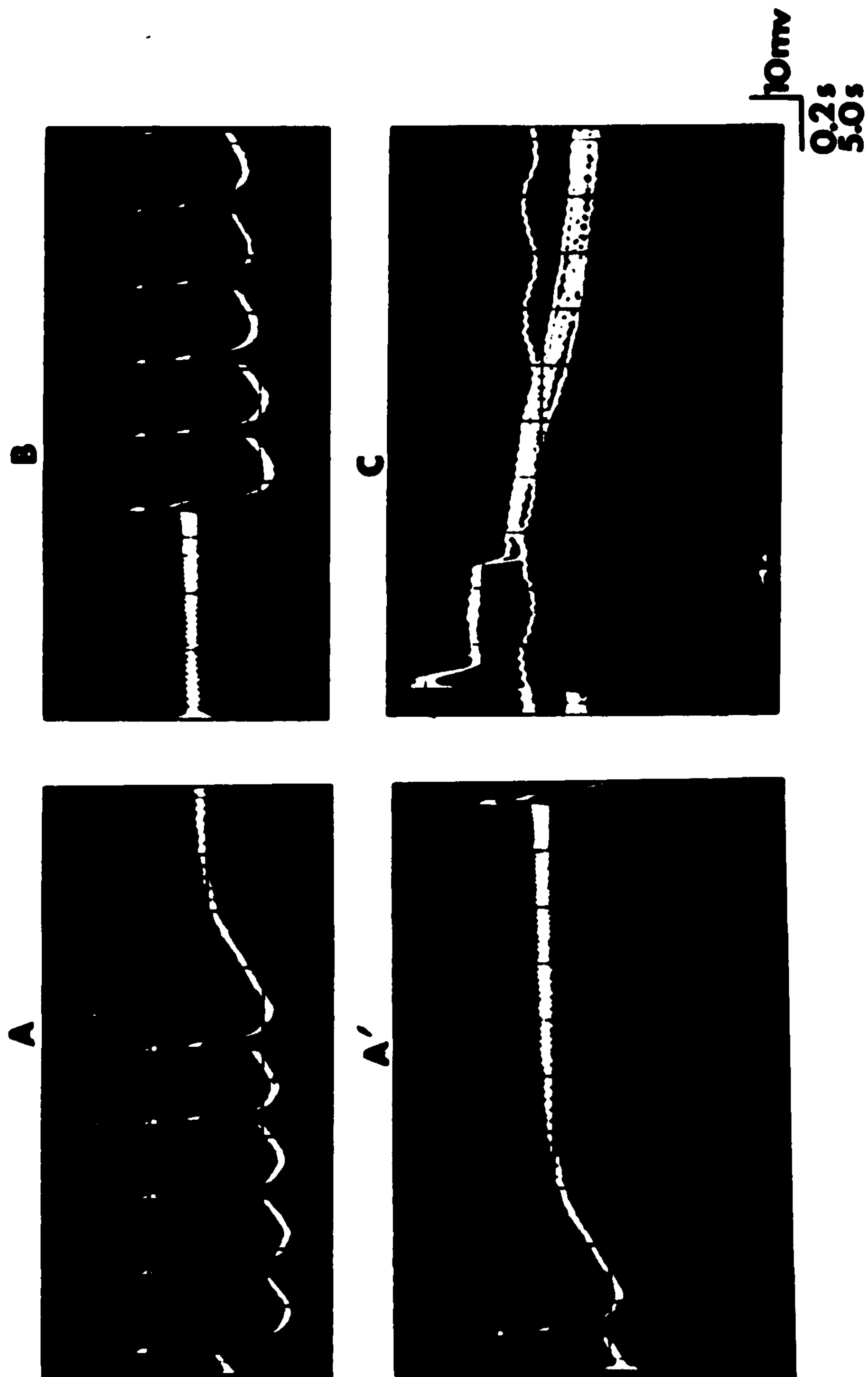


Figure 35

Figure 36

Photoreceptor and L-neuron responses recorded from the same intact nerve preparation under identical conditions of illumination. The records on the left show superimposed responses to 4 consecutive test flashes shown as part of a single sweep recorded at a slower sweep speed to the right. The L-neuron responses were recorded 20 minutes after recording from the photoreceptor. This preparation showed unusually pronounced dark oscillatory activity which was not normally observed in animals with intact ocellar nerves. The oscillations in the photoreceptor appeared approximately 2 seconds following light OFF; those in the L-neuron appeared approximately 1.5 seconds following light OFF.
Stimulus intensity: Log I = -2.
Resting potential: photoreceptor, -42; L-neuron, -48.

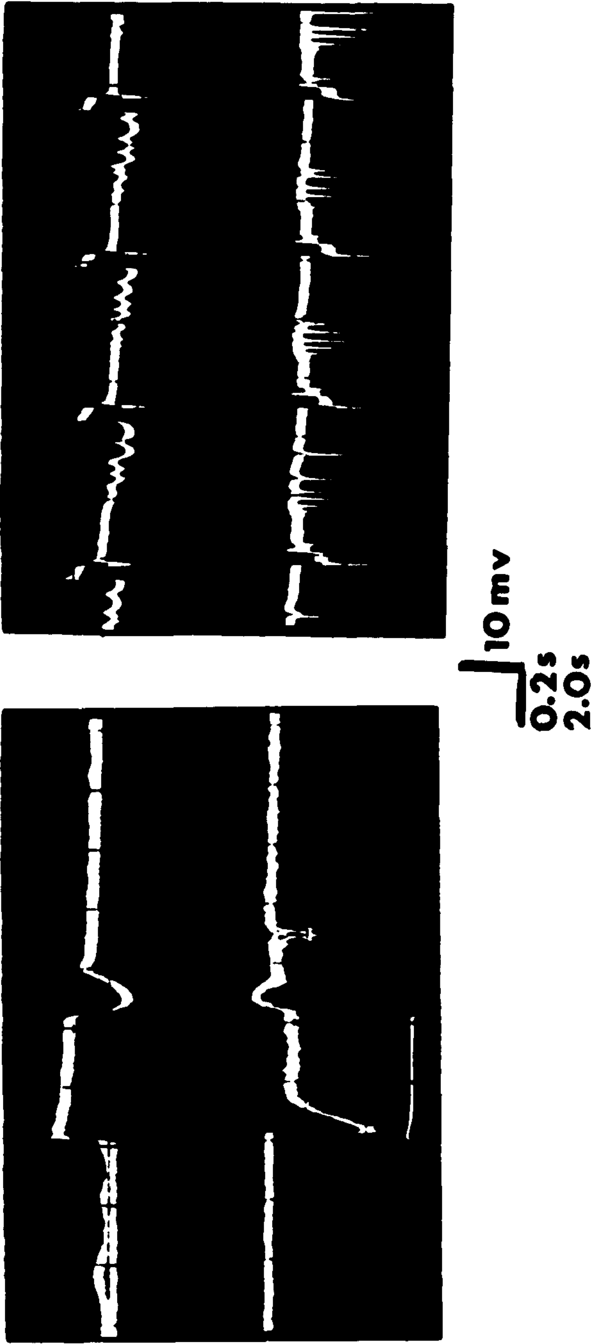


Figure 36

Figure 37

Spontaneous dark oscillations in L-neuron from cut nerve preparation. This is the same cell shown in figure 33.

A: Continuous test flashing was initiated after the preparation was dark adapted for 5 minutes. The waveform and pattern of the spontaneous hyperpolarizations in the dark adapted preparation were similar to that shown during Log I = -6 test flashes in figure 33.

B: The preparation was placed in the dark after continuous test flashing for 9 minutes. The dark adapted oscillatory pattern shown in A (above) was reached after approximately 2 minutes in the dark.

C: Resistance measurements monitored in the dark adapted preparation show an increase in conductance during the spontaneous hyperpolarizations. Conditions for measuring resistance are described in figure 25.

Stimulus intensity for A and B: Log I = -2.

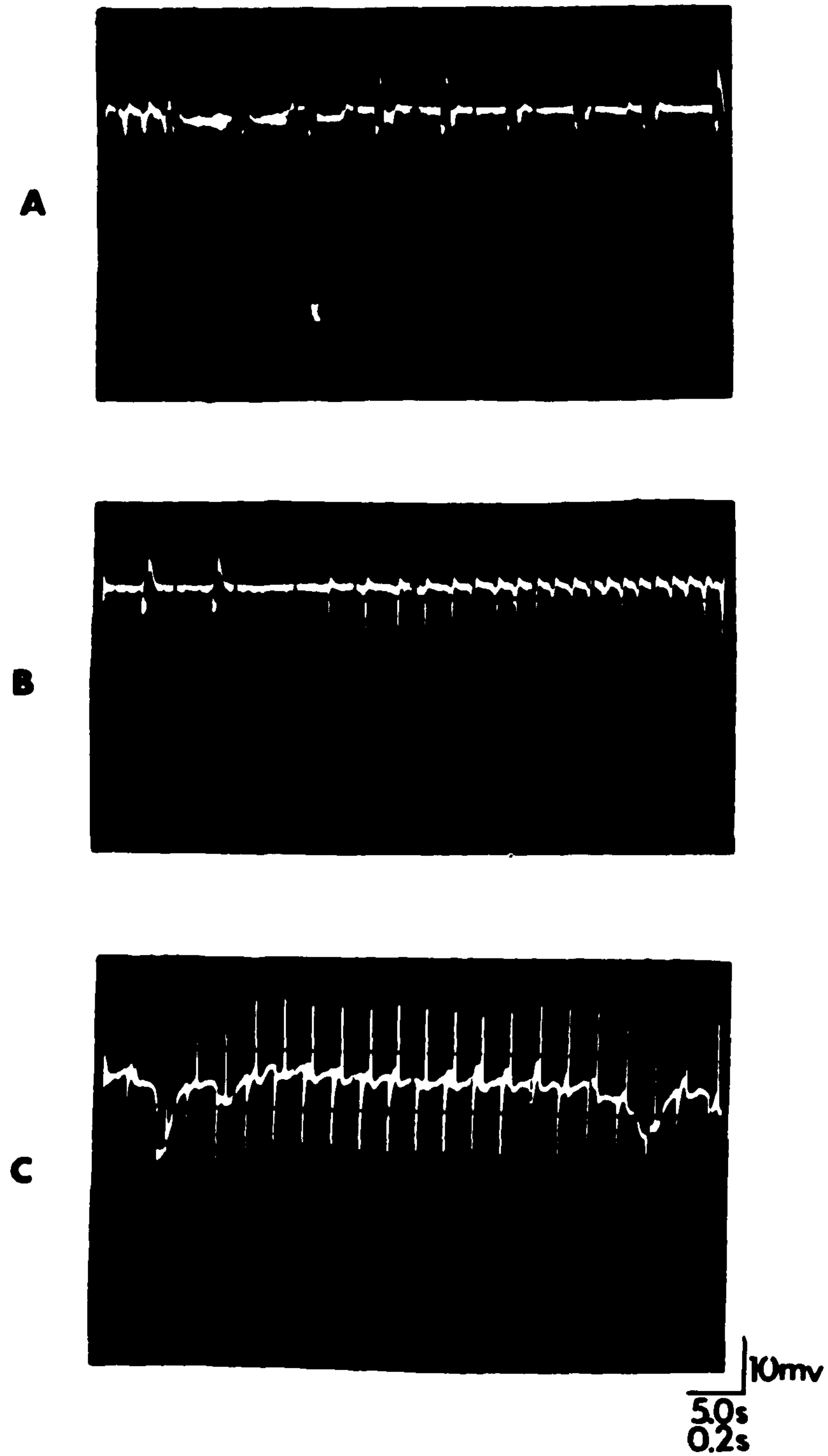


Figure 37

Figure 38

Bistable behavior in photoreceptors from 2 different cut nerve preparations.

A: Spontaneous dark oscillations recorded from cell shown in figure 34 after 6 minutes in the dark.

B: In a different preparation, the pattern of spontaneous dark oscillations (after 4 minutes in the dark) was modified by Log I = -2 test flashes. This is the same cell shown in figures 27 and 76. These oscillations were inhibited by Log I = 0 stimuli (see figure 27).

C: The cell shown in B above was placed in the dark after 4 minutes of continuous test flashing (Log I = -2). The oscillatory pattern in the dark took on more of a spike-like appearance and the large spontaneous hyperpolarizations were reduced in amplitude and frequency. Under conditions of continuous test flashing large hyperpolarization between responses were especially pronounced (see figure 76). The trace in A, and the upper trace in B and C were recorded at the slowere sweep speed (2.0 s).

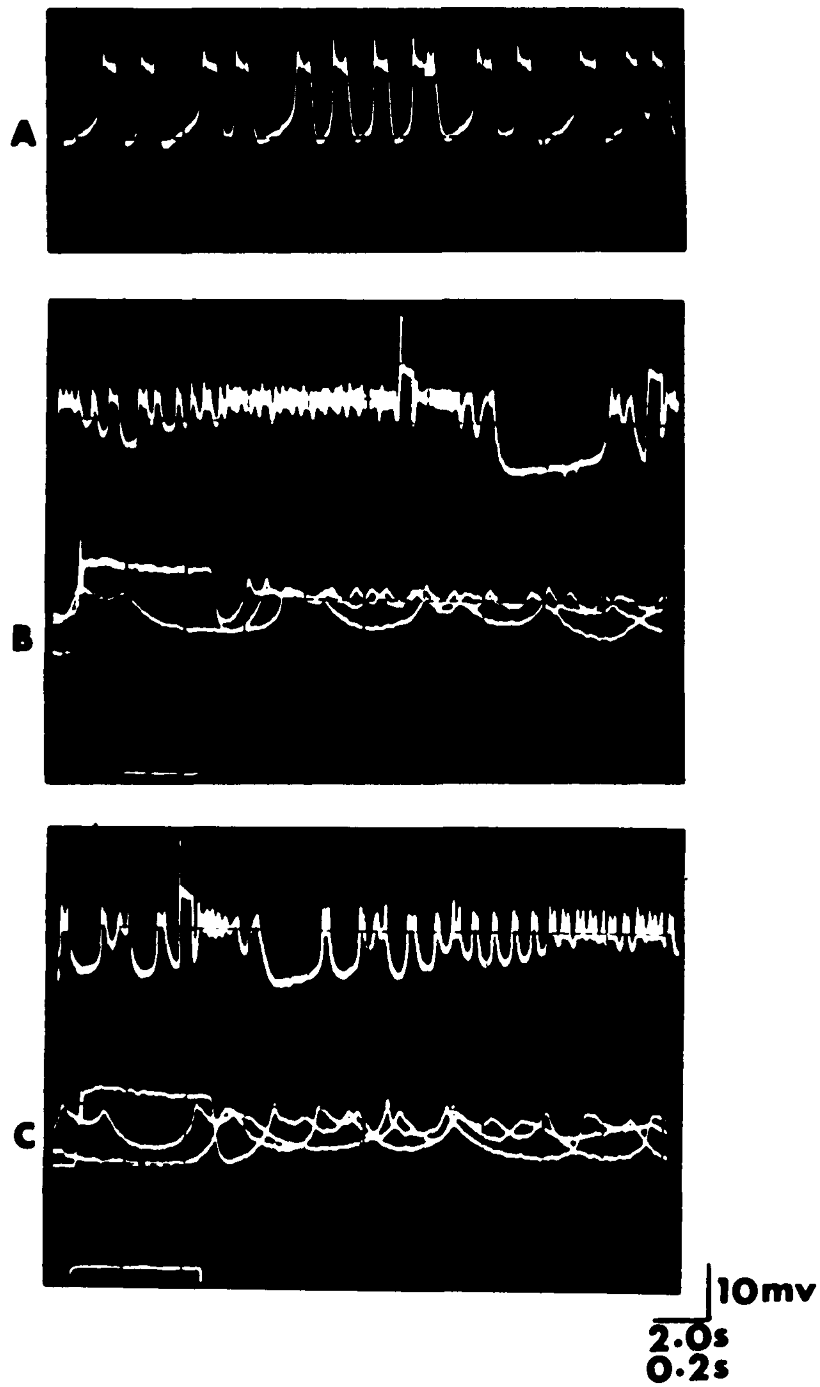


Figure 38

Figure 39

Some examples of bistable behavior in 3 intact nerve preparations after exposure to drugs.

A: Fluctuations in dark potential between flashes during application of 1.6 mM picrotoxin. (This is the same experiment shown in figure 66).

B: The dark potential in this cell (washing after 1 mM picrotoxin) persisted at a more negative level but showed sudden spontaneous positive shifts associated with rapid oscillatory activity. The preparation was placed in the dark after continuous test flashing (Log I = -2). The single light response is shown on the expanded time scale (0.2 sec per division).

C: This photoreceptor developed bistable behavior while washing after curare perfusion. The dark adapted potential in this cell persisted at a more negative level and was stable, but transiently shifted to more positive levels as test flashes of moderate intensity were initiated. Another cell showing similar behavior is shown in figure 57.

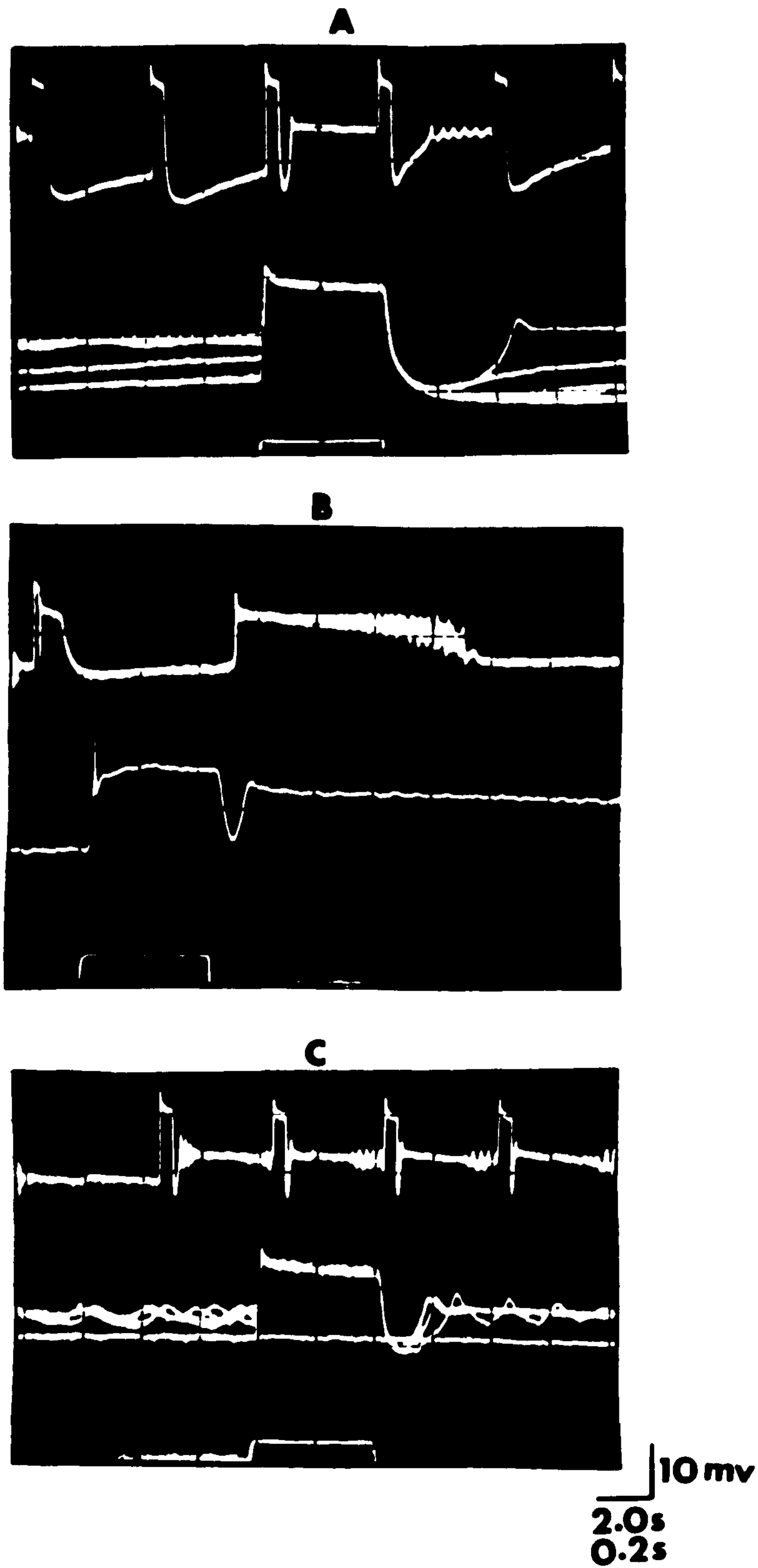


Figure 39

Figure 40

A dim background light appeared to stabilize the interflash potential in bistable cells and the OFF response appeared almost normal. These responses were recorded from two different intact nerve preparations that developed bistable behavior in response to the application of drugs.

A: Photoreceptor washing 15 minutes after the application of 1.6 mM picrotoxin. Turning ON a background light (arrow) depolarized the cell to the positive level reached following recovery from the OFF response.

B: Turning OFF a constant background light caused a 3 mv negative shift in the dark potential of a bistable cell (back to the dark adapted level) but the elimination of the background enhanced the OFF response. This cell had become bistable following exposure to curare. The intensity of the background light was not measured.

Stimulus intensity for A and B: Log I = -2.

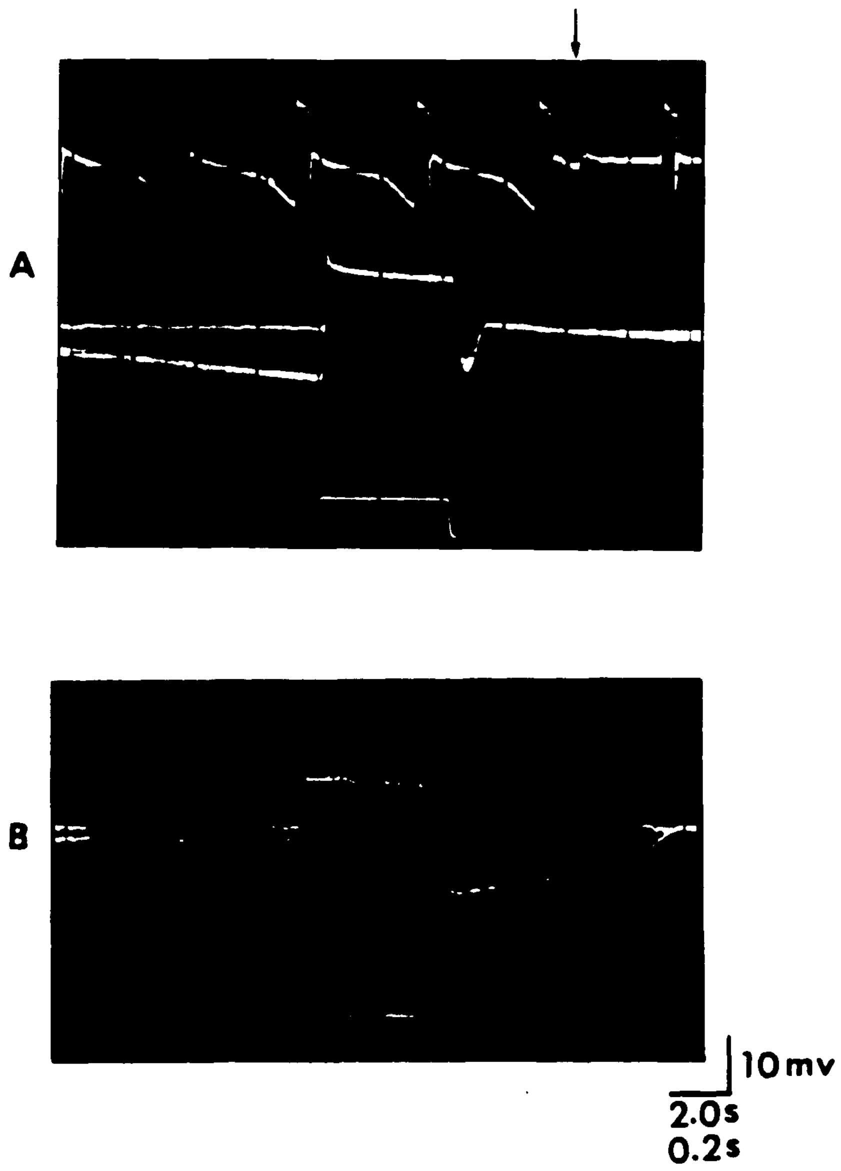


Figure 40

Figure 41

Curare (0.14 mM) blocked the activity of an L-neuron displaying unusually enhanced oscillatory behavior for an intact nerve preparation. Note the change in waveform as the response recovered during the wash period. Under these stimulus conditions the spontaneous dark hyperpolarizations did not return. During the application of curare the dark potential depolarized 5 mv. After 16 minutes of washing, the dark potential had shifted 10 mv more positive than before curare perfusion. When washing was initiated there was a small (-2 mv) transient negative shift in the dark potential.
Stimulus intensity: Log I = -2.
Resting potential: -48 mv (before curare perfusion).

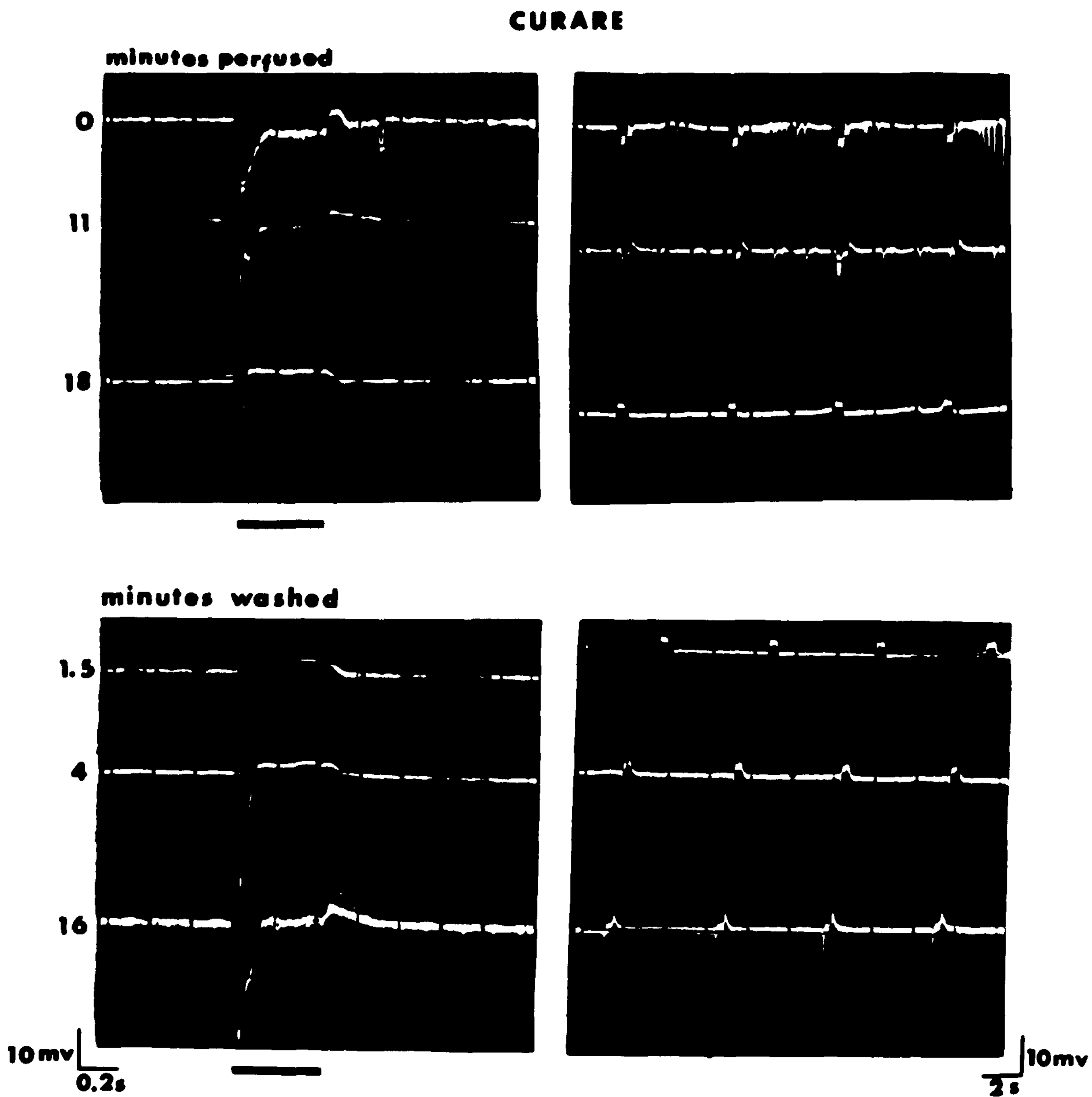


Figure 41

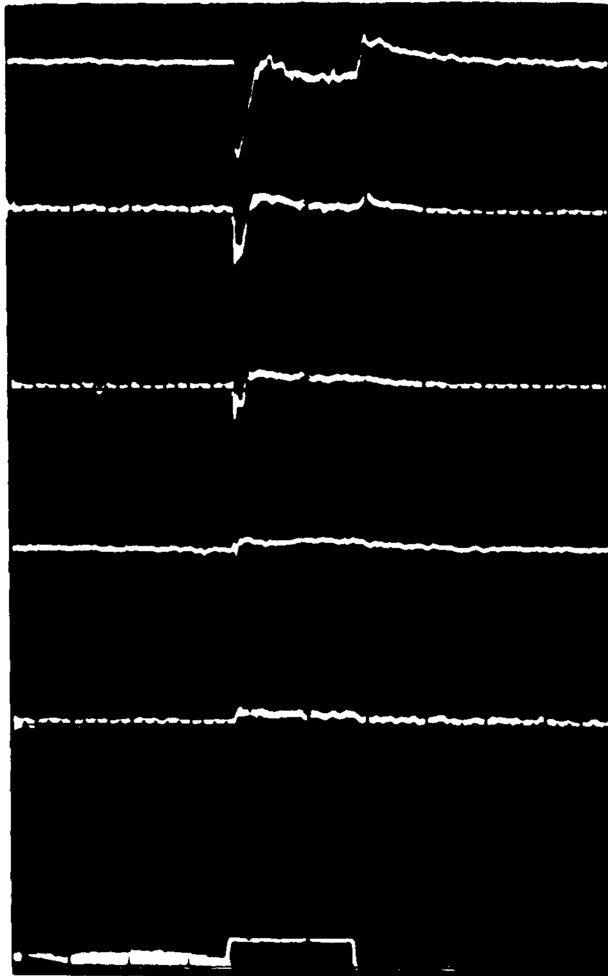
Figure 42

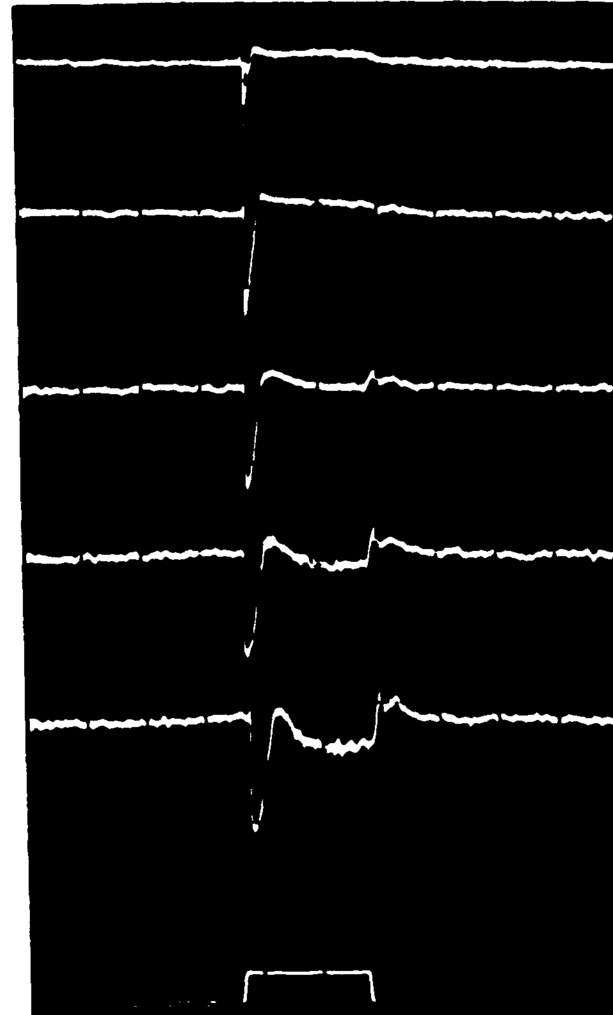
Curare (1.4 mM) reversibly blocked the L-neuron response in an intact nerve preparation. During perfusion, the dark potential depolarized 1-2 mv and continued to shift positive (up to 6 mv) during the wash period. The cell was impaled after losing the cell shown in figure 44. The preparation was washing 1 hour when the perfusion (shown here) was initiated. The data in this figure is represented in Graphs V, VI and VII.

Stimulus intensity: Log I = -2.

Resting potential: -47 mv.

minutes perfused CURARE minutes washed





10 mv
0.2 s

Figure 42

Figure 43

This figure shows 3 and 4 superimposed responses stored over a period of 20 and 30 minutes respectively during the curare experiment shown in the preceding figure (figure 42). These responses were recorded at the times indicated in figure 42, but may not be the identical responses.

A: Three superimposed responses corresponding to "before", "perfused 3 min" and "washed 10 min" as shown in figure 42. The two responses with the more negative dark potential before light ON correspond to the period before curare perfusion and after 3 minutes of exposure to the drug, and the more positive response was recorded after 10 minutes of washing.

B: Same responses shown in A above plus 1 more response recorded after 20 minutes of washing. The two responses with the more positive dark potential correspond to 10 and 20 minutes of washing. The dark potential had shifted approximately 5 mv more positive after 10 minutes of washing and was 6-7 mv more positive after 20 minutes of washing; before the electrode was withdrawn from the cell. There was a 47 mv positive D.C. shift in potential when the electrode was withdrawn (indicating a resting potential of -47 mv at this time). The data in this figure is represented in Graphs V, VI and VII.

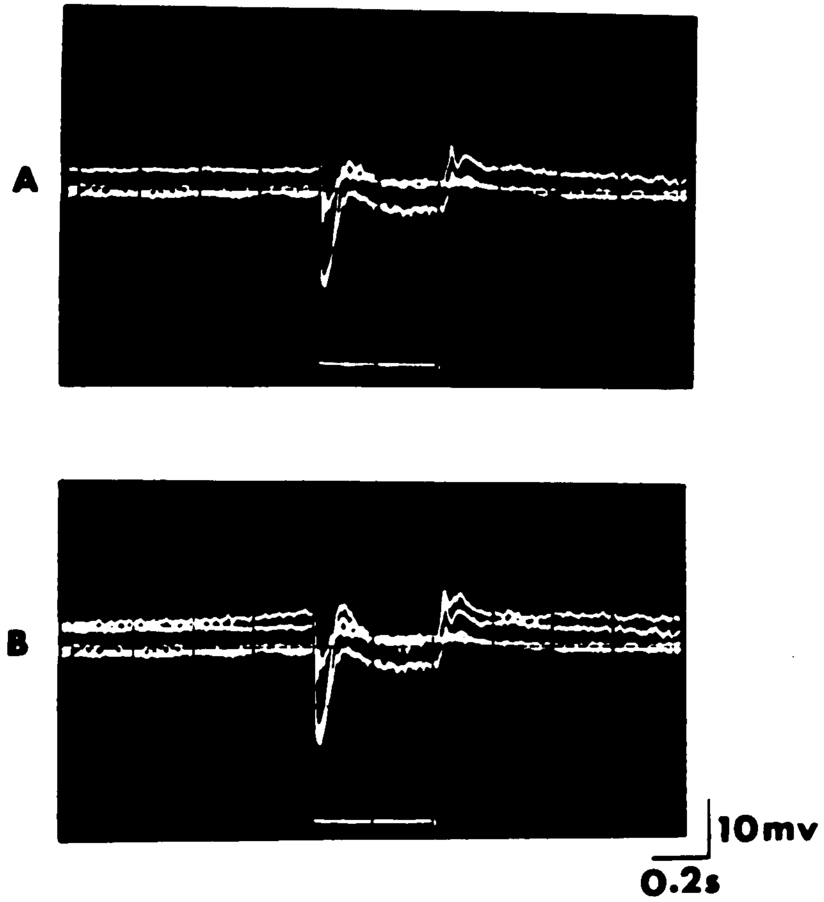


Figure 43

Figure 44

Effect of 0.5 mM curare on L-neuron from cut nerve preparation. An intensity-response series from this cell is shown in figure 23. Under stimulus conditions of continuous test flashing the OFF-response lost its oscillatory appearance (see figure 23). The light response was blocked in less than 5 minutes and did not recover completely before the cell was lost. The resting potential had depolarized 8 mv during the application of curare and was 10 mv more positive before the cell was lost.

Stimulus intensity: Log I = -3.

Resting potential: -48 mv (before curare).

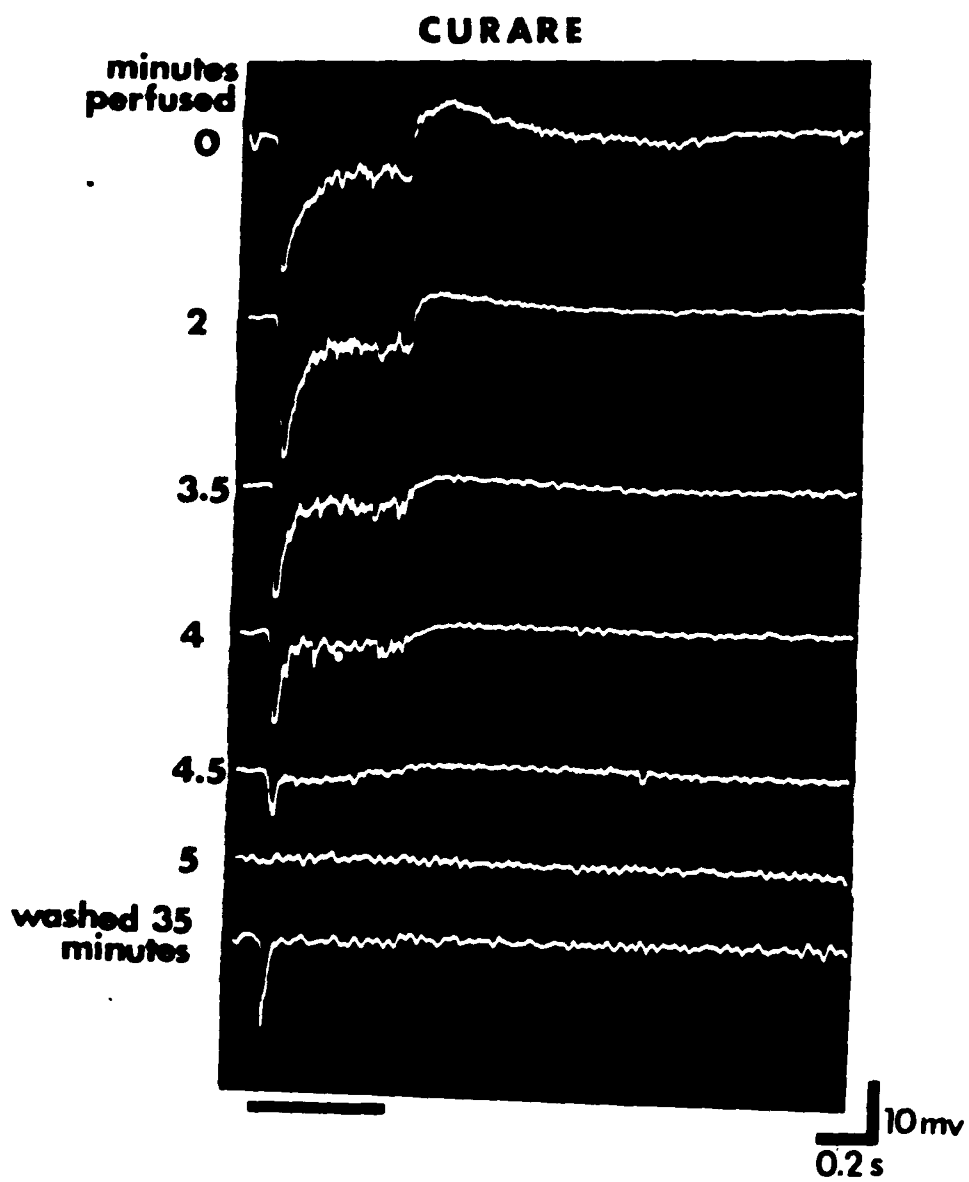


Figure 44

Figure 45

Effect of 0.5 mM curare on L-neuron response from cut nerve preparation. The light response was blocked in less than 3 minutes and only partially recovered to the original waveform after a prolonged wash. The resting potential appeared to depolarize 10 mv by the start of the wash period and did not seem to change significantly thereafter. However, in this experiment there is some doubt about the change in dark potential because there was transient drift in the baseline of the oscilloscope trace.

Stimulus intensity: Log I = -2.

Resting potential (before curare): -60 mv.

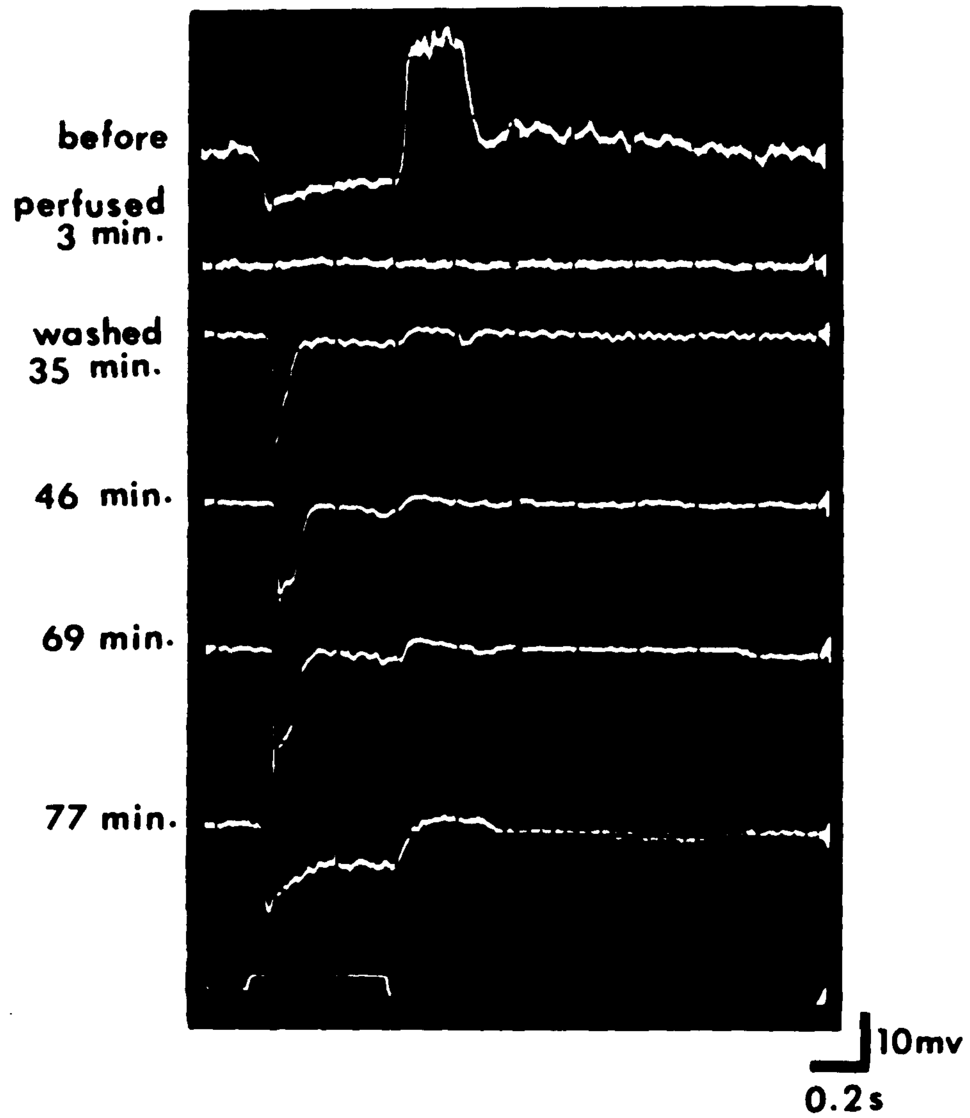


Figure 45

Figure 46

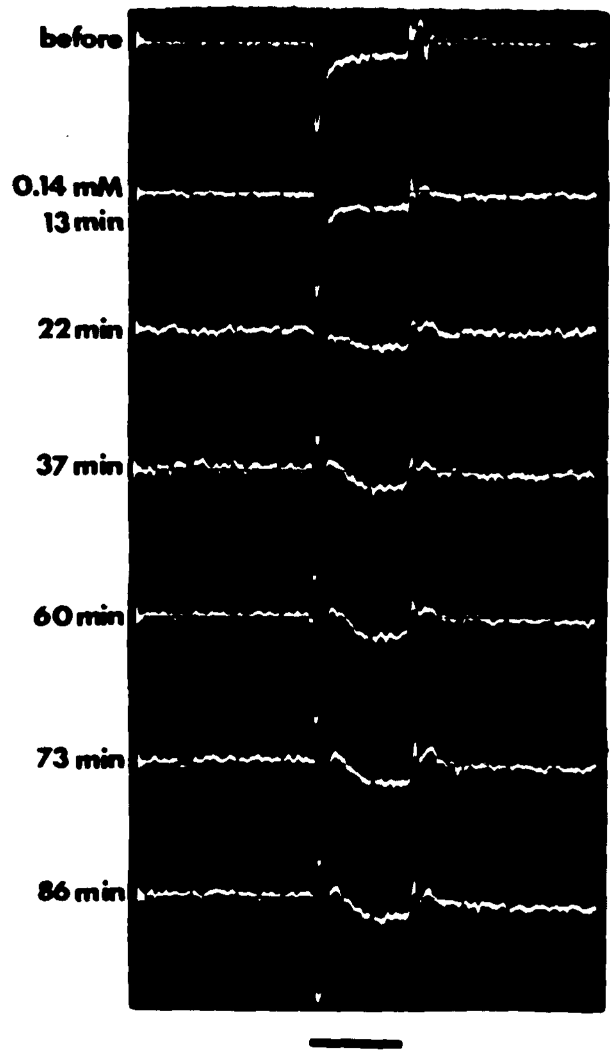
Effect of two doses of curare on the L-neuron response from an intact nerve preparation. The lower concentration of curare (0.14 mM) did not block the light response but the waveform changed in a characteristic manner. Subsequent perfusion with a higher concentration of curare (1.4 mM) abolished the light response after 28 minutes, leaving a small residual depolarization. The response started to recover after a brief wash but was lost before recovery was complete.

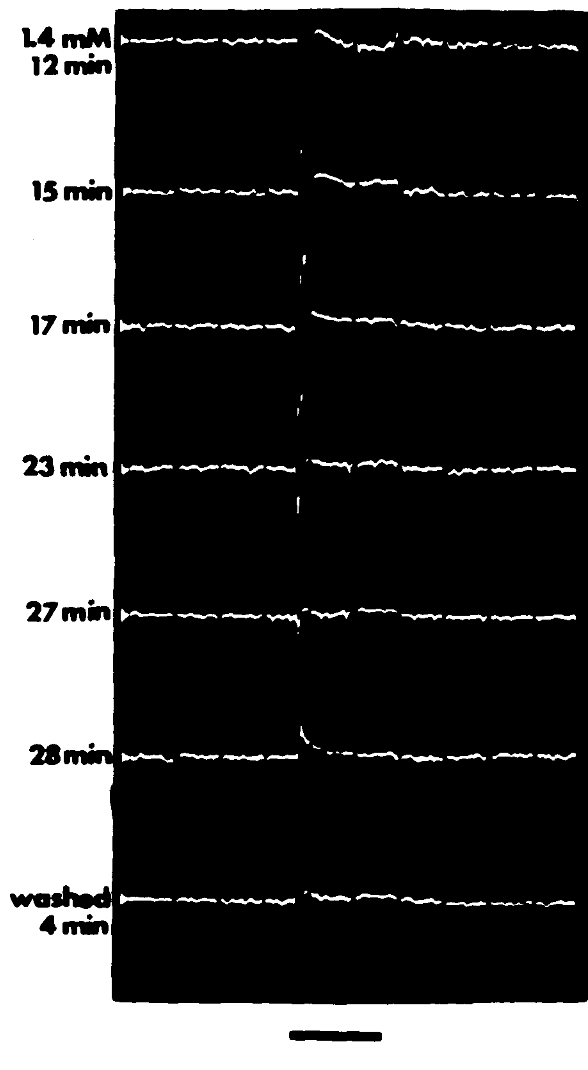
The resting potential in this cell shifted approximately 10 mv more positive during the first 20 minutes of curare (0.14 mM) perfusion and remained at that level for the remainder of the experiment. The resting potential measured -49 mv when the cell was impaled. The data in this figure is represented in Graphs VIII, X, XI, XII, XIII, XIV, XV, XVI.

A second L-neuron dendrite was impaled after the preparation had been washing for 50 minutes and had a normal appearing light response (see figure 42).

Stimulus intensity: Log I = -2.

CURARE





10 mv
0.2 s

Figure 46

Figure 47

This figure shows up to 6 superimposed responses stored over a period of 2 hours during the curare experiment shown in figure 46. The dark potential in the L-neuron shifted to a more positive level during the application of curare. The superimposed responses shown here were recorded at the approximate times indicated in figure 46 but they are not necessarily the identical responses.

A: Two responses before and after 13 minutes perfusion with 0.14 mM curare. The dark potential had shifted 9 mv more positive and the OFF response was less phasic.

B: Three superimposed responses before, and during 13 and 73 minutes of exposure to 0.14 mM curare. The dark potential shifted 12 mv more positive during prolonged exposure to the drug, although continued application of curare for an additional hour caused only an additional 2 mv positive shift in the dark potential before light ON. Note the change in the waveform of the response.

C: Five superimposed responses before and during the application of 0.14 and 1.4 mM curare respectively. The additional responses (compared to B) show two responses after exposure to 1.4 mM curare for 2 minutes (not shown in figure 46), and 12 minutes respectively. The dark potential prior to light-ON did not change significantly during the application of the more concentrated solution, although the potential following light-OFF was slightly more positive than during the application of the less concentrated solution.

(continued...)

Figure 47 (continued)

D: Six superimposed responses stored over a period of two hours, before and during perfusion with 0.14 and 1.4 mM curare respectively. The 12 mv positive shift in dark potential after 73 minutes exposure to 0.14 mM curare did not change significantly during the application of the more concentrated solution. The last response stored in this record was recorded after 23 minutes of exposure to 1.4 mM curare. Although the response was almost completely blocked at this time, the 12 mv positive shift in dark potential had not changed. When the cell was lost after 12 minutes of washing, the ON-transient had increased in amplitude. The data in this figure is represented in Graphs VIII-XII.

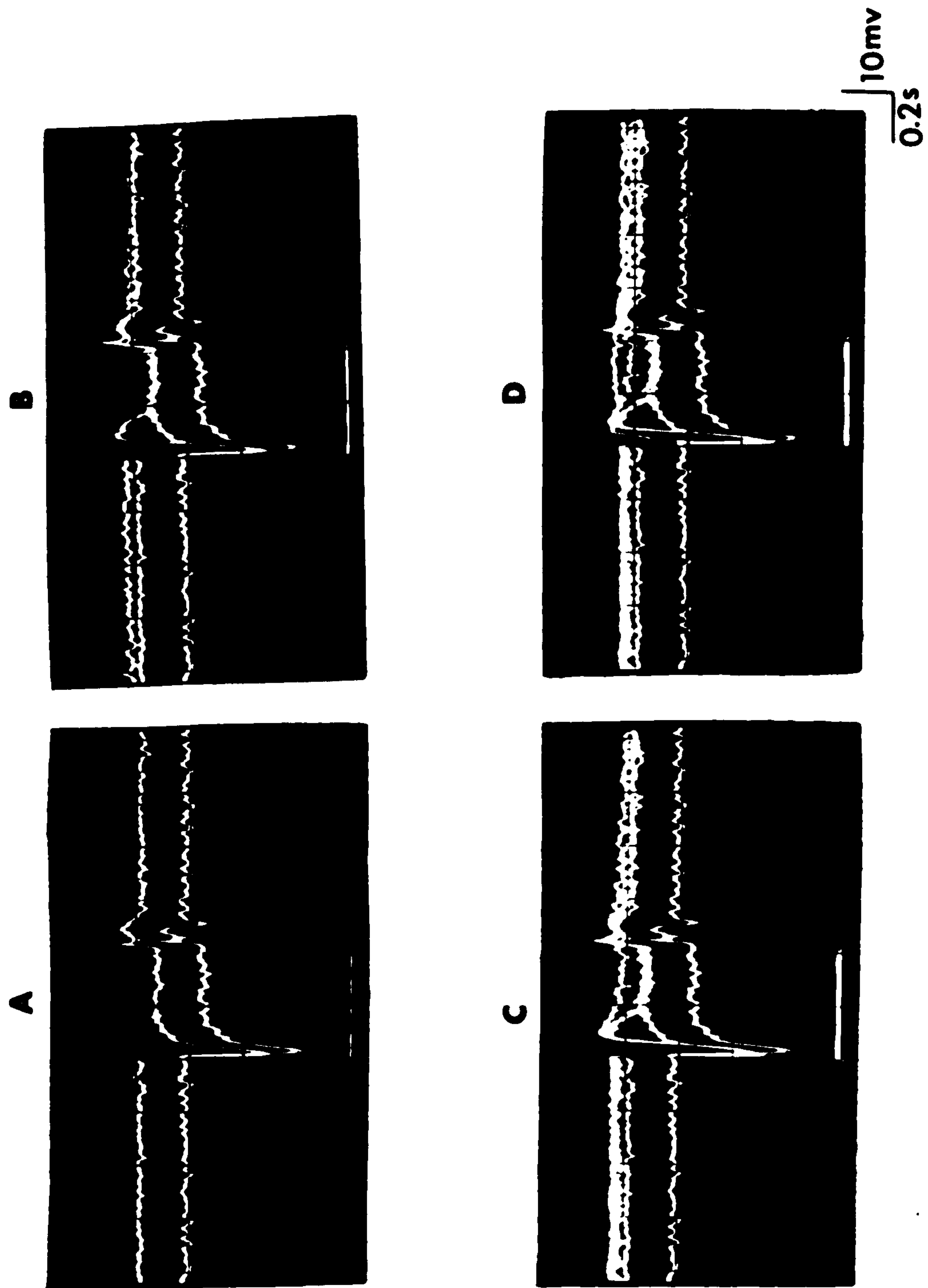


Figure 47

Figure 48

Intensity-response series from L-neuron recorded over a period of 2 hours before and during the application of two concentrations of curare. This is the same cell shown in figures 46 and 47.

A: Before curare.

B: Perfused with 0.14 mM curare for 38 minutes.

C: Perfused with 0.14 mM curare for 88 minutes.

D: Perfused with 1.4 mM curare for 14 minutes.

The change in waveform during the application of curare seems to vary with stimulus intensity. The data in this figure is represented in Graphs XIII-XVI. Intensity-response series after the response was completely blocked (28 min., fig. 48) is not shown.

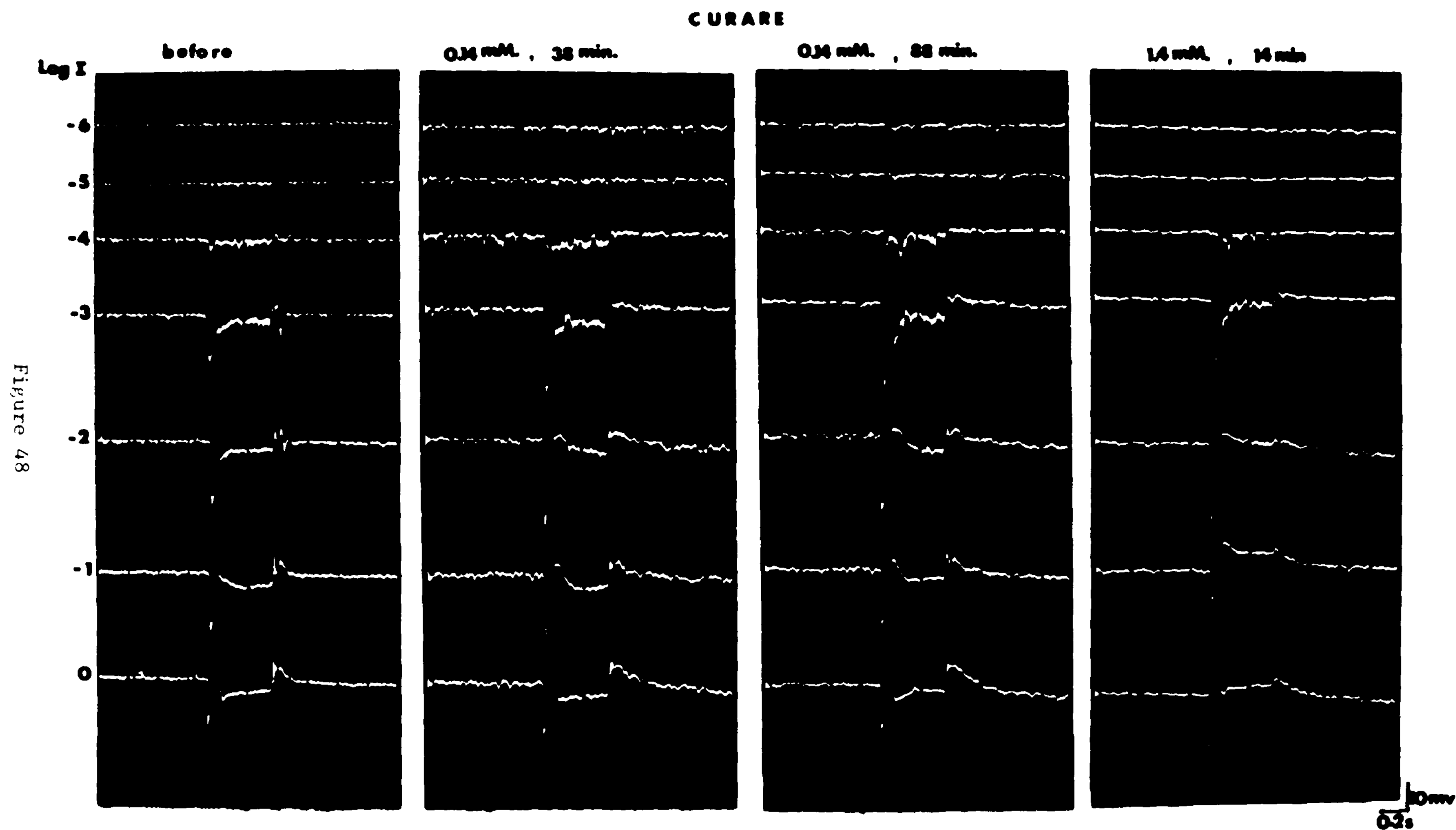


Figure 48

Figure 48

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Figure 49

These records show L-neuron responses recorded over a period of 45 minutes during the application of 0.14 mM curare. The cell was lost before the response was completely blocked. After 15 minutes perfusion the ON-transient became larger in magnitude and the depolarizing wave preceding the cutback from ON-transient to plateau became more prominent. The sustained portion of the light response became about 4 mv more positive. The changes were accompanied by an 8 mv positive shift in the dark potential. With continued perfusion, the dark potential gradually shifted more negative (toward the original level before curare perfusion) while the OFF-response and plateau portion of the light response gradually disappeared. Each of the 4 separate responses (before and during curare) are shown superimposed in the lower record. The level to which the ON-transient hyperpolarized at light-ON had not changed, although the OFF response and plateau portion of the light response disappeared. The data from this experiment is represented in Graphs XVII-XIX.

Stimulus intensity: Log I = -2.

Resting potential (before losing cell): -40 mv.

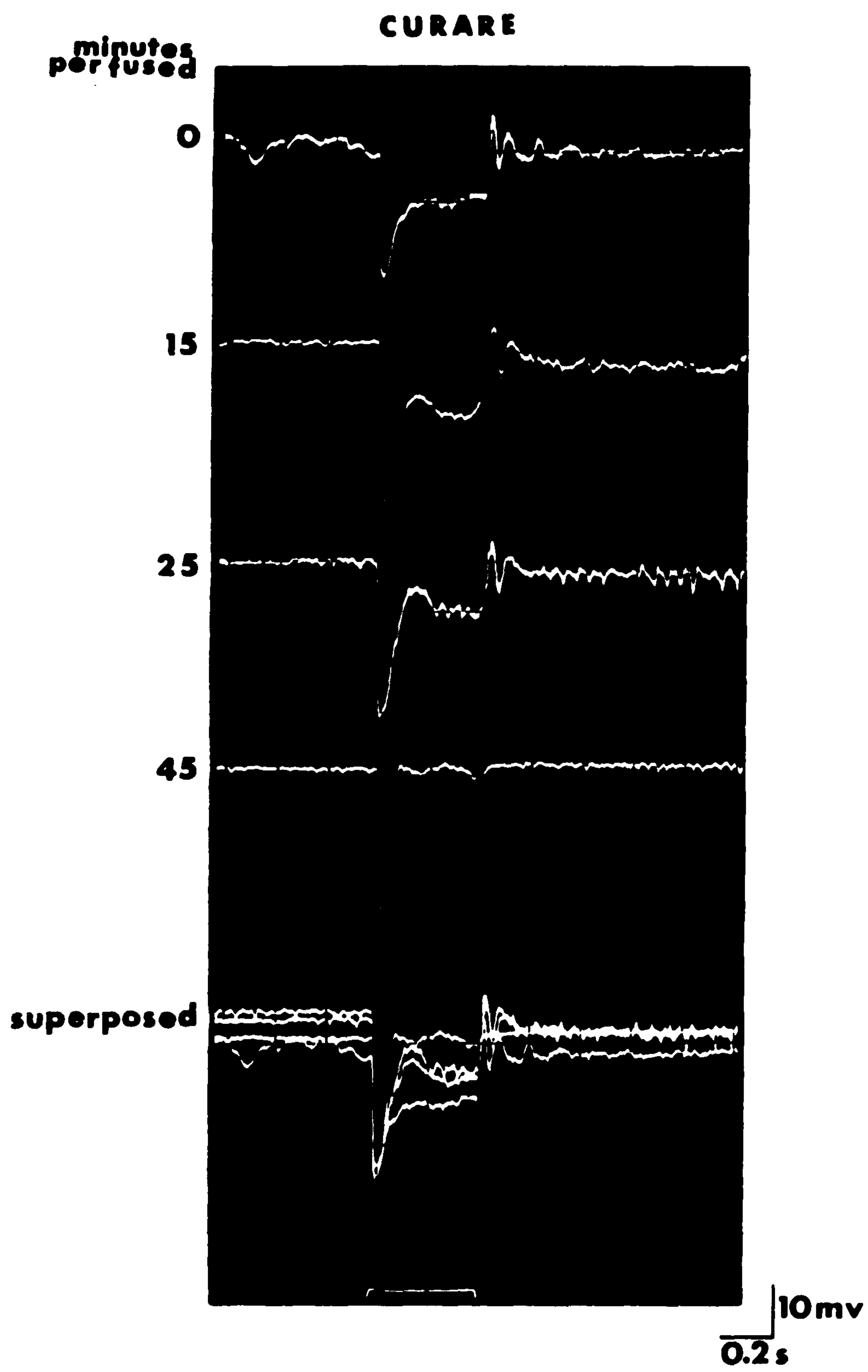


Figure 49

Figure 50

A small, long latency residual depolarization was often observed after the L-neuron response was blocked by curare. These records were recorded from the curare experiment shown in figure 42.

A: Intracellular L-neuron response during 3-4 minute exposure to 1.4 mM curare, just before the ON-transient was blocked.

B: Intracellular L-neuron response 5 minutes after application of 1.4 mM curare. The hyperpolarizing ON transient was blocked and a small, residual depolarization can be seen.

C: Extracellular response (electroretinogram) recorded after the electrode was withdrawn from the cell. This response was recorded after the preparation had been washing 20 minutes and the L-neuron response had recovered (see figure 42). An electroretinogram was not detected in the synaptic region of the ocellus when an L-neuron was lost while the response was abolished in a curare experiment. (For example, an electroretinogram was not seen in the experiment shown in figure 46 just after the cell was lost.)

These findings suggest that extracellular currents, not necessarily generated by L-neurons, are correlated with the residual depolarization recorded intracellularly during curare block.

Stimulus intensity: All, Log I = -2.

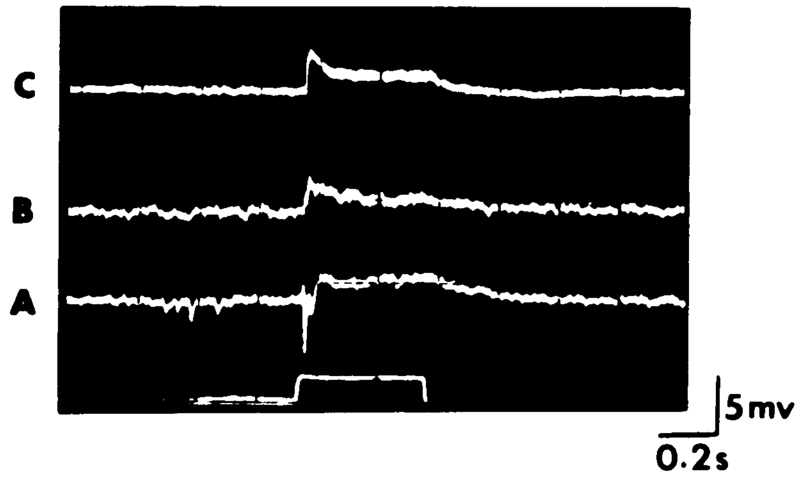


Figure 50

Figure 51

Effect of 1.4 mM curare on photoreceptor responses from intact nerve preparation. Each record shows superimposed responses to 3 consecutive test flashes. During the application of curare the OFF response gradually increased in duration and decreased slightly in amplitude. Partial recovery was seen during the wash period, before the cell was lost. The data in this figure is represented in Graphs XX and XXI.

Stimulus intensity: Log I = -2.

Resting potential: -58 mv.

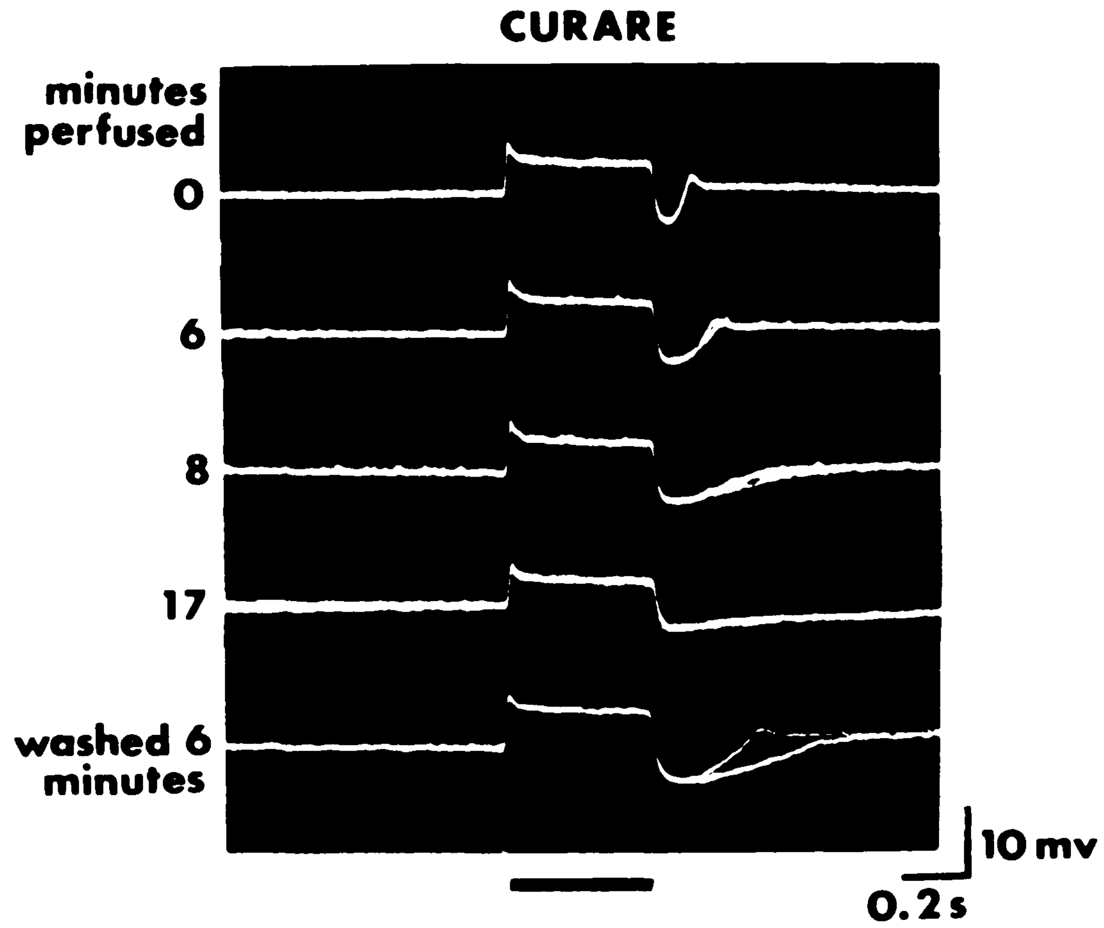


Figure 51

Figure 52

The OFF response in a photoreceptor from a cut nerve preparation was blocked during the application of 0.5 mM curare. The upper two records show superimposed responses to 4 consecutive test flashes. The lower record shows two superimposed responses before, and 5 minutes after, the application of the drug. The OFF response from this cell did not undershoot the dark potential before drug perfusion. After the application of curare, the depolarizing component of the OFF response (recovery phase) was eliminated. This preparation was washed for 26 minutes before the cell was lost but the waveform did not change. Later in the same experiment another photoreceptor was impaled after the preparation had been washing 40 minutes. The latter cell showed an OFF response and bistable behavior. The data from this experiment is represented in Graph XXII.

Stimulus intensity: Log I = -2.

Resting potential: -58 mv.

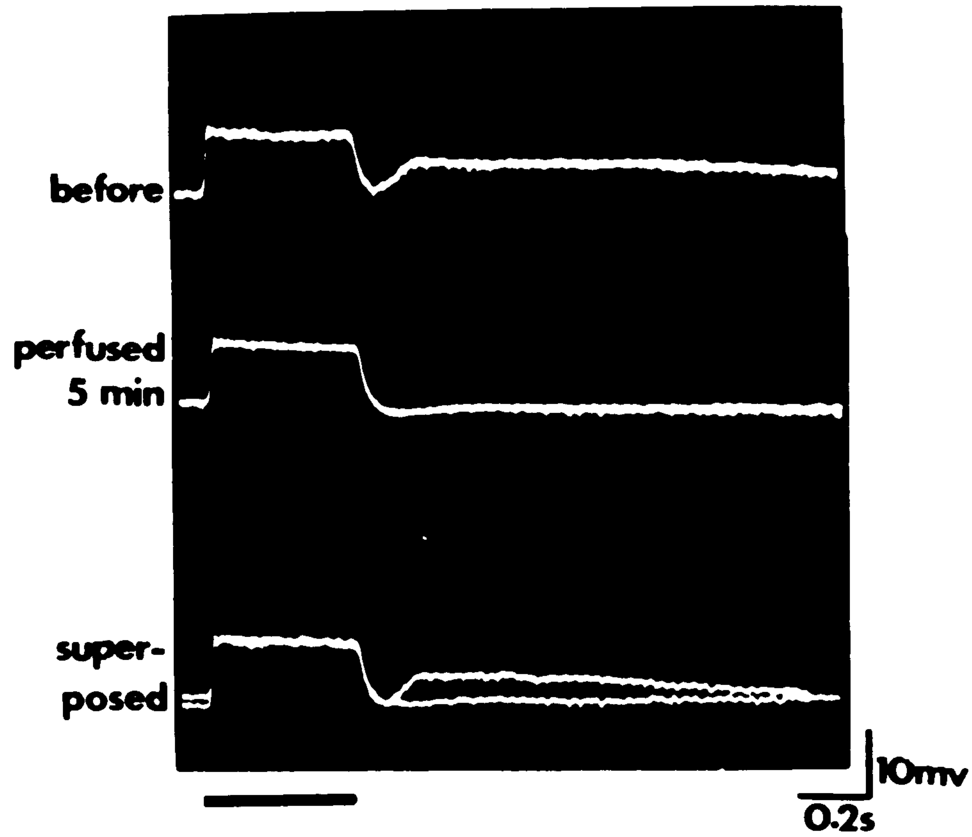


Figure 52

Figure 53

Curare (0.5 mM) blocked the spontaneous dark oscillatory activity in a photoreceptor from a cut nerve preparation. The records on the left show superimposed responses to 4 consecutive test flashes shown as part of a single trace recorded at the slower sweep speed to the right.

Stimulus intensity: Log I = -2.

Resting potential before curare (most positive level): -58 mv.

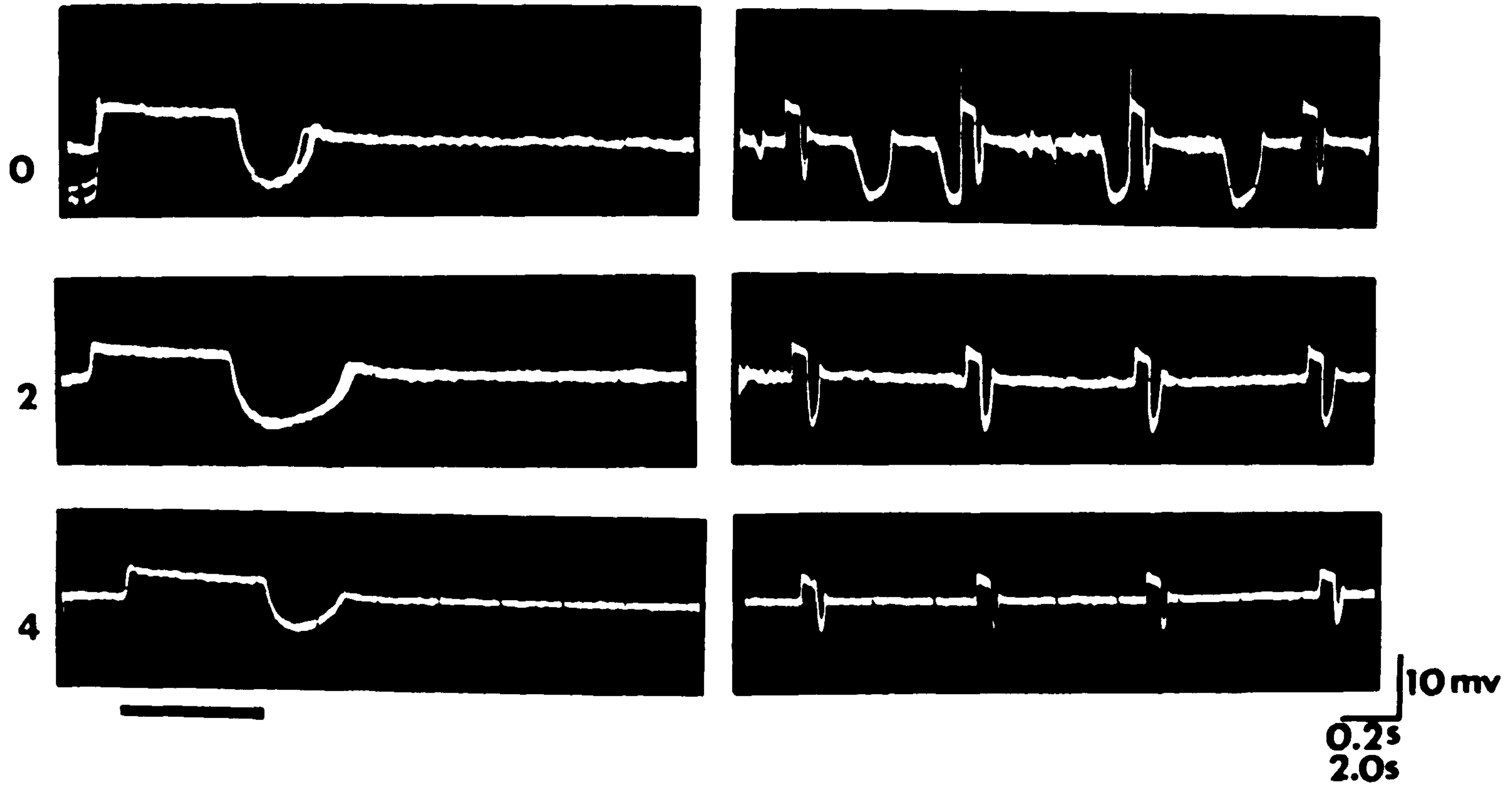


Figure 53

Figure 54

Effect of eserine sulfate on the photoreceptor response from an intact nerve preparation. This cell displayed small sinusoidal oscillations in the dark which are normally observed in approximately 15% of intact nerve photoreceptors. At this light intensity (Log I = -1) they appeared approximately 2 seconds following light OFF (see figure 19). Perfusion with 0.3 uM eserine had a two-phased effect on the receptor OFF response. Initially, the OFF transient increased slightly in duration and decreased slightly in amplitude, thus causing the OFF response to appear less phasic. In this respect, the initial effect of eserine on the receptor OFF response was similar to a bright test flash because the OFF response is normally less prominent following brighter stimuli (see figures 2-4). With continued perfusion the OFF response increased in amplitude as well as duration. The second effect of eserine also mimicked the effect of light because it was shown that long duration stimuli tend to enhance the receptor OFF response (see figures 9-14). Furthermore, eserine and light had a similar effect in inhibiting the spontaneous oscillatory activity between test flashes (see figures 2, 27, 19). As shown above, during the application of eserine the small sinusoidal oscillations disappeared, but reappeared, increased in amplitude and duration during wash. In this experiment the resting potential remained stable at -45 mv. The effect of a higher concentration of eserine on the response of this cell is shown in the next figure (55). The data from this experiment is represented in Graphs XXIII, XXV, XXVI and XXVII.

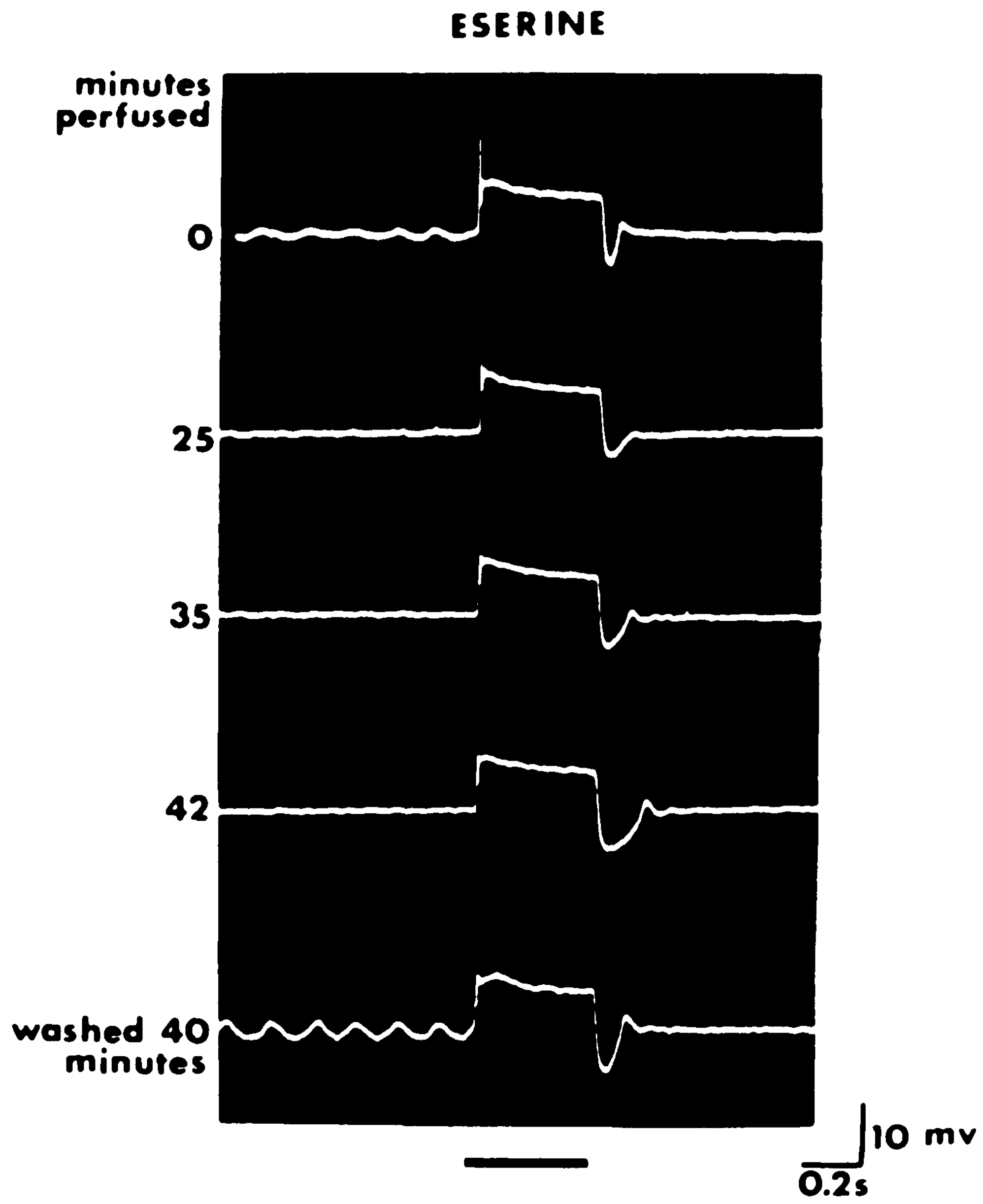


Figure 54

Figure 55

The cell shown in the preceding figure (figure 54) was treated with 1.5 uM eserine. The dark oscillations were blocked and the OFF response changed as before (similar to that seen during perfusion with 0.3 uM eserine) except that the OFF response continued to increase in amplitude and duration during the wash period. The OFF response was especially enhanced at lower light intensities (not shown). The data from this experiment is represented in Graphs XXIV, XXVI and XXVII.

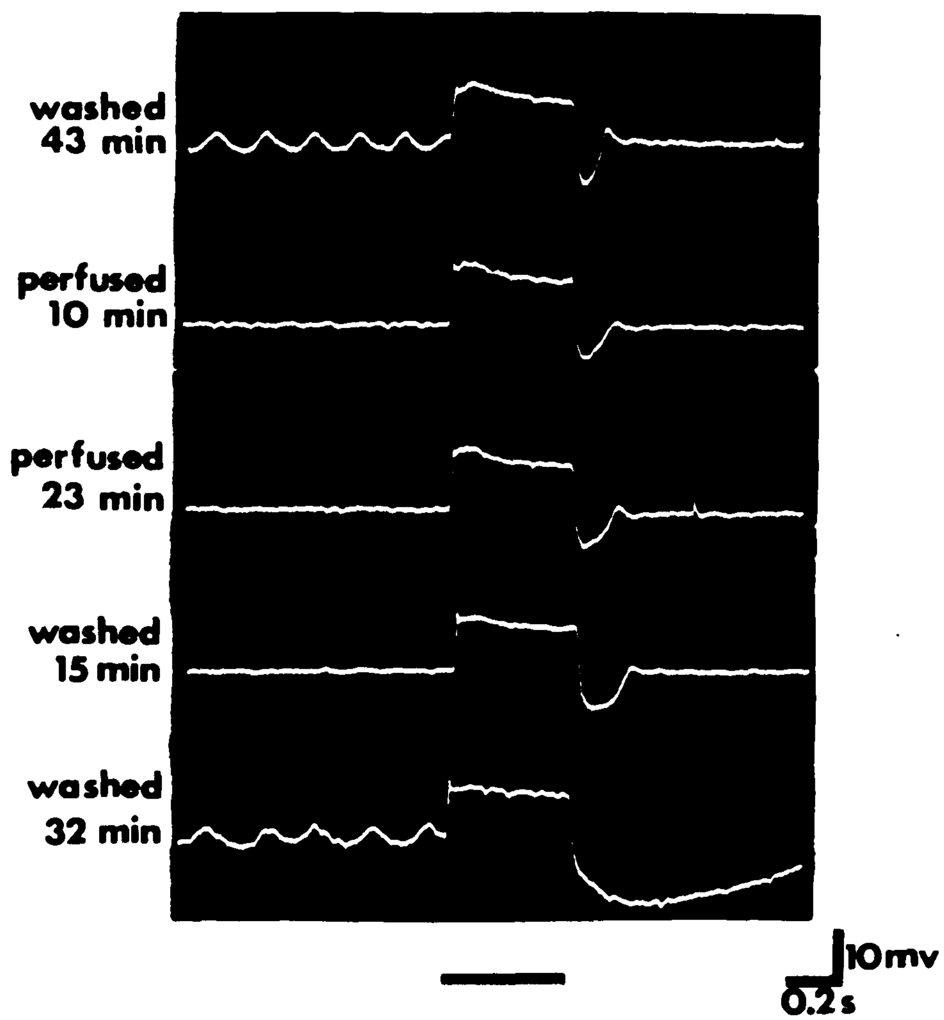


Figure 55

Figure 56

Photoreceptor response from intact nerve preparation during eserine perfusion (2 uM). Each record shows superimposed responses to 4 consecutive test flashes. During exposure to eserine the OFF response became smaller and less phasic. Continued perfusion caused the OFF response to increase in amplitude and duration, and during the wash period the OFF response became irregular. After 27 minutes of washing a decline in resting potential was seen, and the cell was lost a short time later. This cell did not display any type of dark oscillatory behavior.

Stimulus intensity: Log I = -2.

Resting potential: -48 mv.

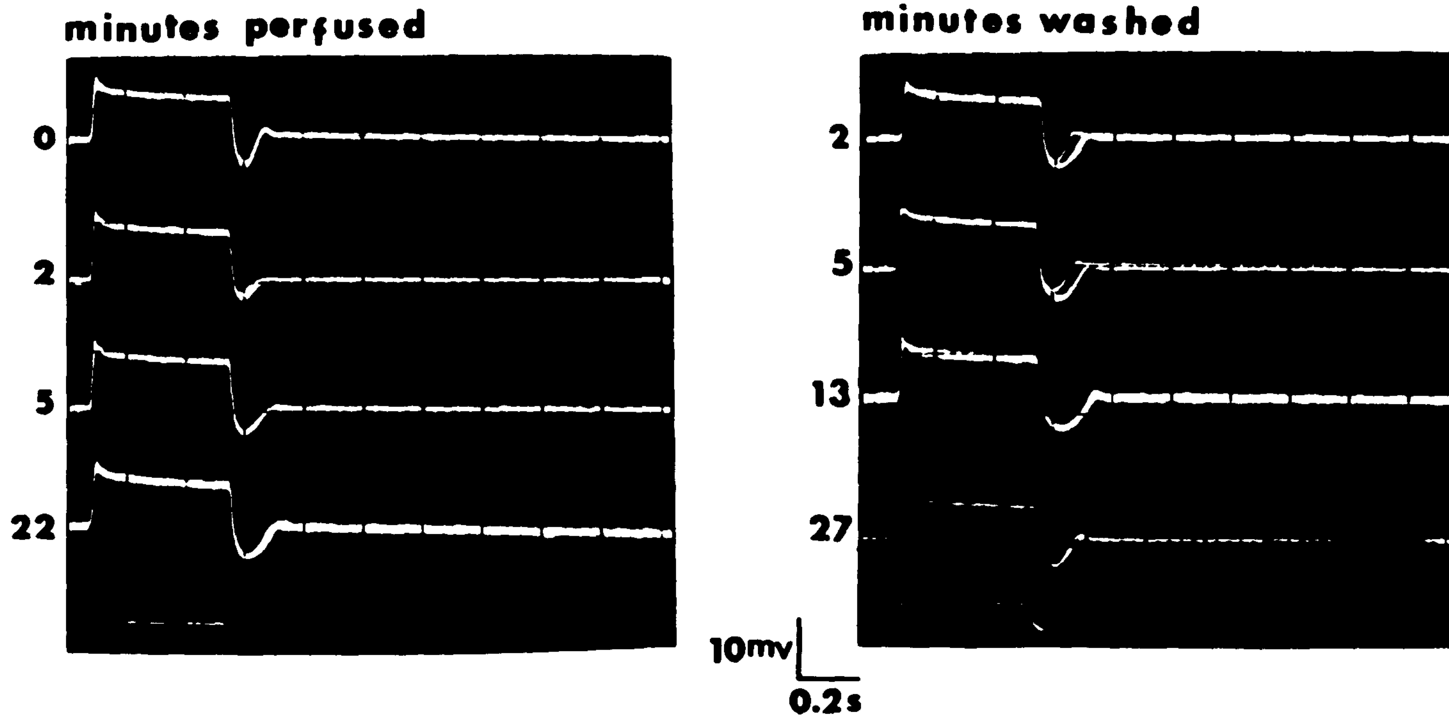


Figure 56

Figure 57

Effect of 1.5 uM eserine on a photoreceptor that developed bistable behavior while washing after curare perfusion (later in the experiment shown in figure 51). The bistable condition developed over a period of 45 minutes following the application of curare and persisted for an additional hour before eserine was applied. All the responses on the slow sweep are shown superimposed on the faster sweep immediately below.

Top left: Before eserine; this cell was quiet in the dark but displayed oscillatory and bistable behavior as test flashes of moderate intensity (Log I = -2) were initiated.

Bottom left: Before eserine; the interflash waveform reached this relatively steady state after 4 minutes of continuous test flashing.

Top right: After 4 minutes of perfusion, 1.5 uM eserine stabilized the dark potential at the more positive level shown in the preceding records. The preparation was dark adapted following the third light response in this record, but dark oscillations did not reappear.

Lower right: This record shows 5 superimposed oscilloscope sweeps sampled over a period of 12 minutes before and during eserine perfusion. This figure shows 2 dark adapted traces and 3 light responses, indicated by the arrows in figure 57. Note the presence of two distinct dark equilibrium potentials.

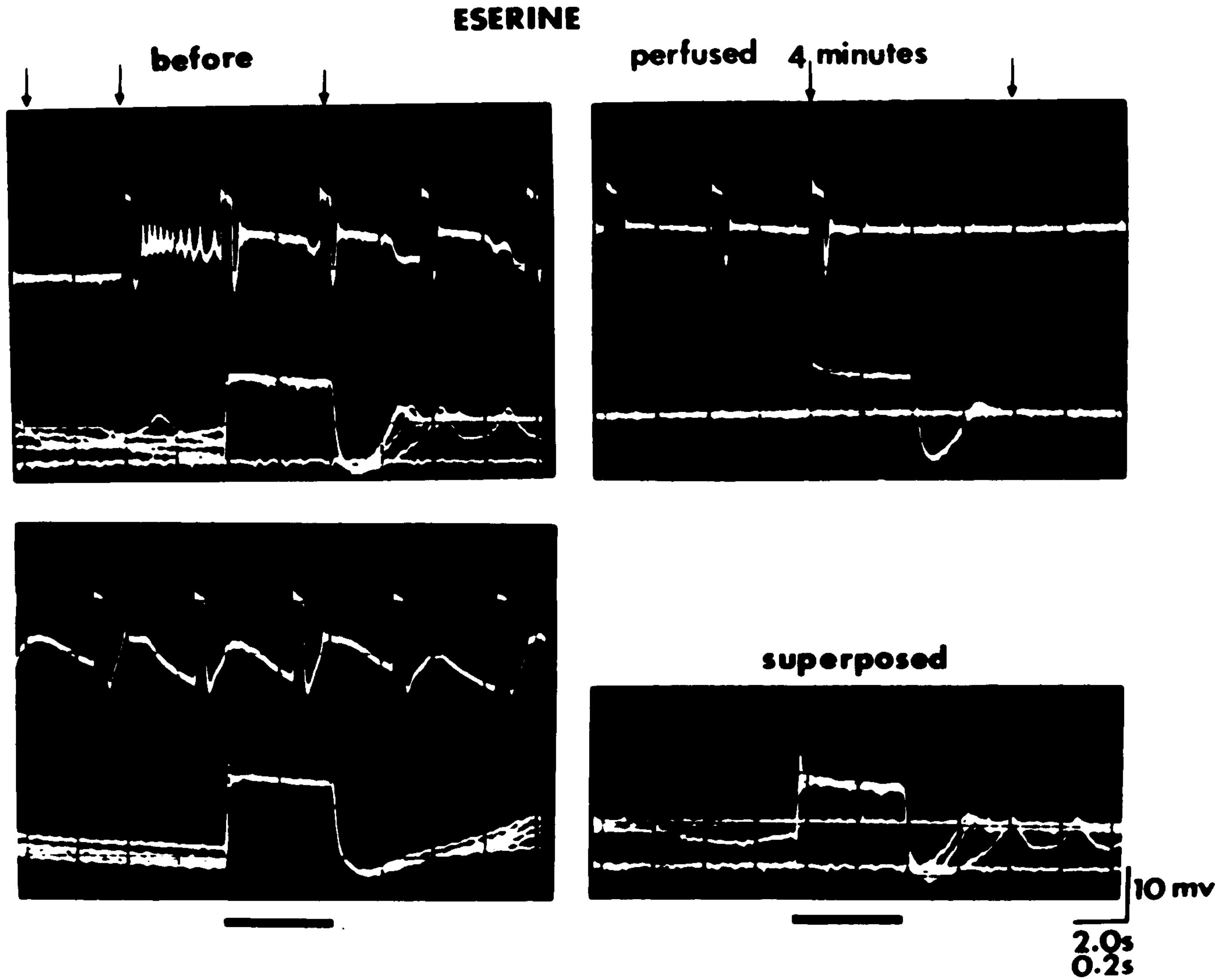


Figure 57

Figure 58

Effect of continued eserine perfusion (1.5 uM) on a cell that had previously displayed bistable behavior. This is the same cell shown in the preceding figure (figure 57). Each record shows three or four superimposed responses to consecutive test flashes (Log I = -2). Some of the records show the resting potential when the cell was placed in the dark. Continued eserine perfusion caused a two-phased change in the OFF response as described earlier in figures 54 and 56. However, the OFF response continued to increase in amplitude and duration during the wash period and did not recover. The dark adapted potential and the potential between test flashes remained stable. Subsequent perfusion with curare reduced the OFF response (figure 59). The data in this figure is represented in Graphs XXVIII and XXIX.

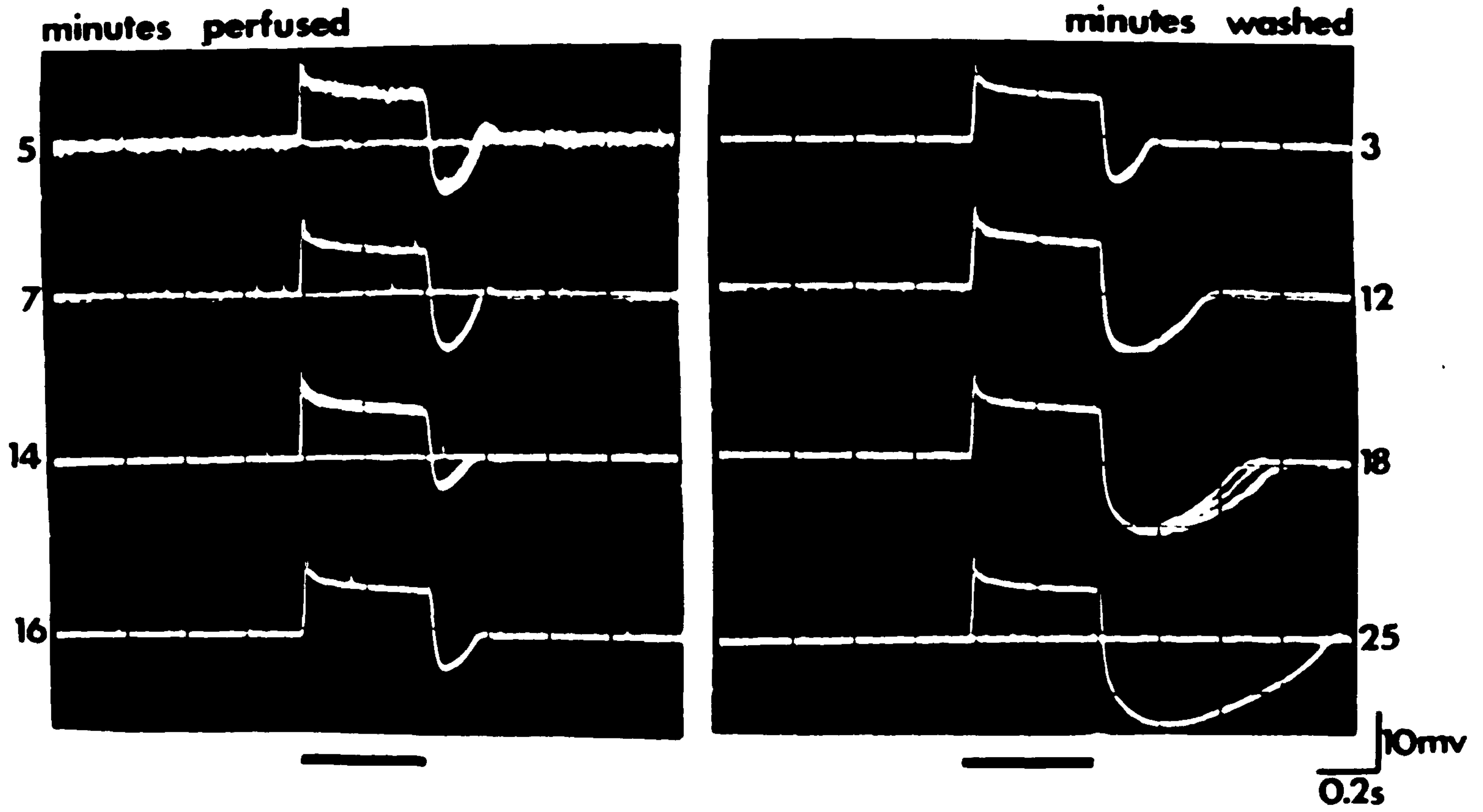


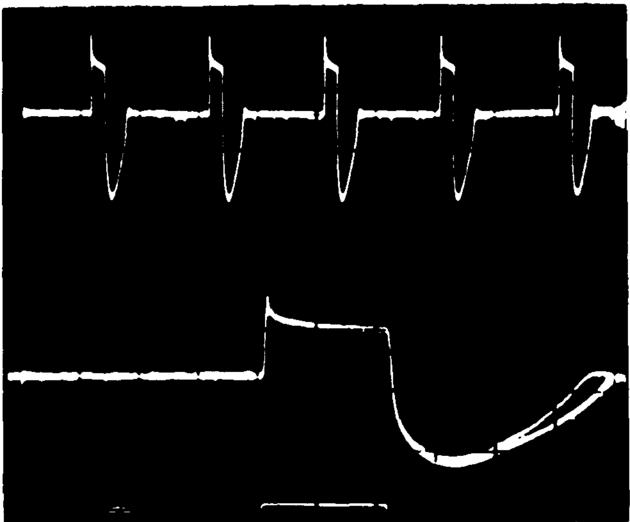
Figure 58

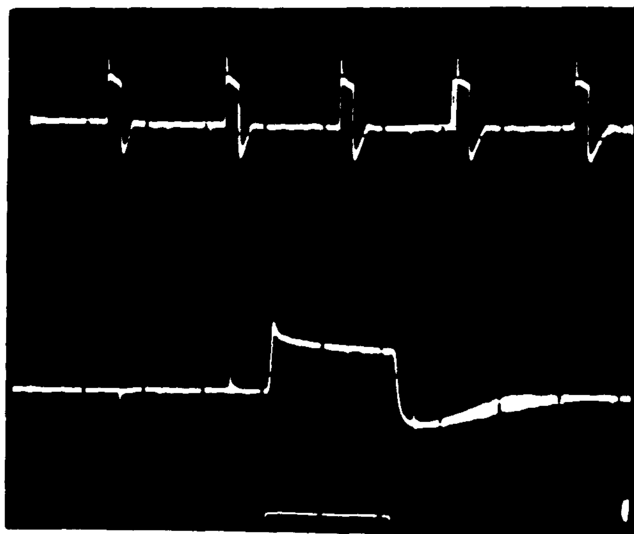
Figure 59

These records are a continuation of the same experiment shown in the preceding 2 figures (figures 57, 58). The records on the left show the photoreceptor response following the application of 1.5 uM eserine. The preparation had been washing about 25 minutes and the OFF response continued to increase in amplitude and duration during this period. The records on the right show the receptor response during exposure to 1.4 mM curare for 8 minutes. The OFF response was dramatically reduced, similar to that seen earlier in figure 51. An intensity-response series recorded after these records were taken showed a large post-illumination hyperpolarization following Log I = 0 test flashes (not shown). This cell was lost several minutes later. The small deflections seen in the curare records are due to droplets of perfusate falling on top of the preparation.

Stimulus intensity: Log I = -2.

Resting potential: -40 mv.





10 mv
2.0s
0.2s

Figure 59

Figure 60

This record shows the change in waveform of the L-neuron response in the presence of a dim background light. Top: The L-neuron response to a Log I = -2 test flash before the background light was turned on. Middle: L-neuron response to the same intensity test flash (I = -2) recorded 30 seconds after turning on a dim background light. Note the reduction in the amplitude of the ON transient and sustained portion of the light response. These same two responses are shown superimposed in the lower record. Note that the "dark" potential in the presence of dim background illumination was only slightly more negative than the dark potential in the absence of background illumination. After 2 minutes of exposure to background illumination the light response became larger although the dark potential did not change (not shown). In some experiments, sustained bright illumination did shift the sustained potential to more negative levels, but the qualitative change in waveform to a test flash was similar to that shown here. The intensity of the background illumination was not measured.

Resting potential: -43 mv.

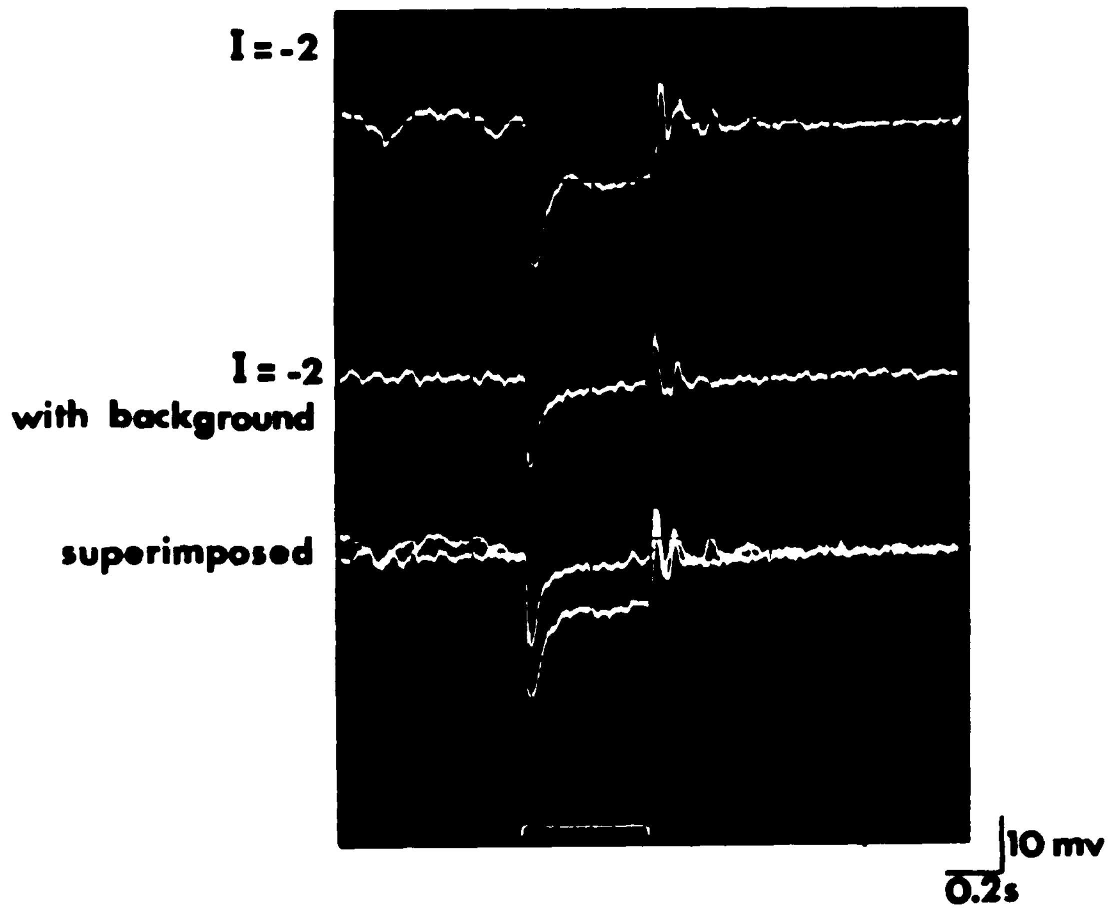


Figure 60

Figure 61

Effect of 2 uM eserine on the L-neuron response from an intact nerve preparation. During perfusion the ON-transient and sustained portions of the light response were reduced. The change in waveform resembled the change observed in the presence of a constant background light (see figure 60). The OFF response was not reduced to the same extent as the ON-transient and plateau. The dark potential depolarized 8 mv during exposure to eserine and the waveform and dark potential recovered after 30 minutes of washing.

Stimulus intensity: Log I = -2.

Resting potential: -48 mv.

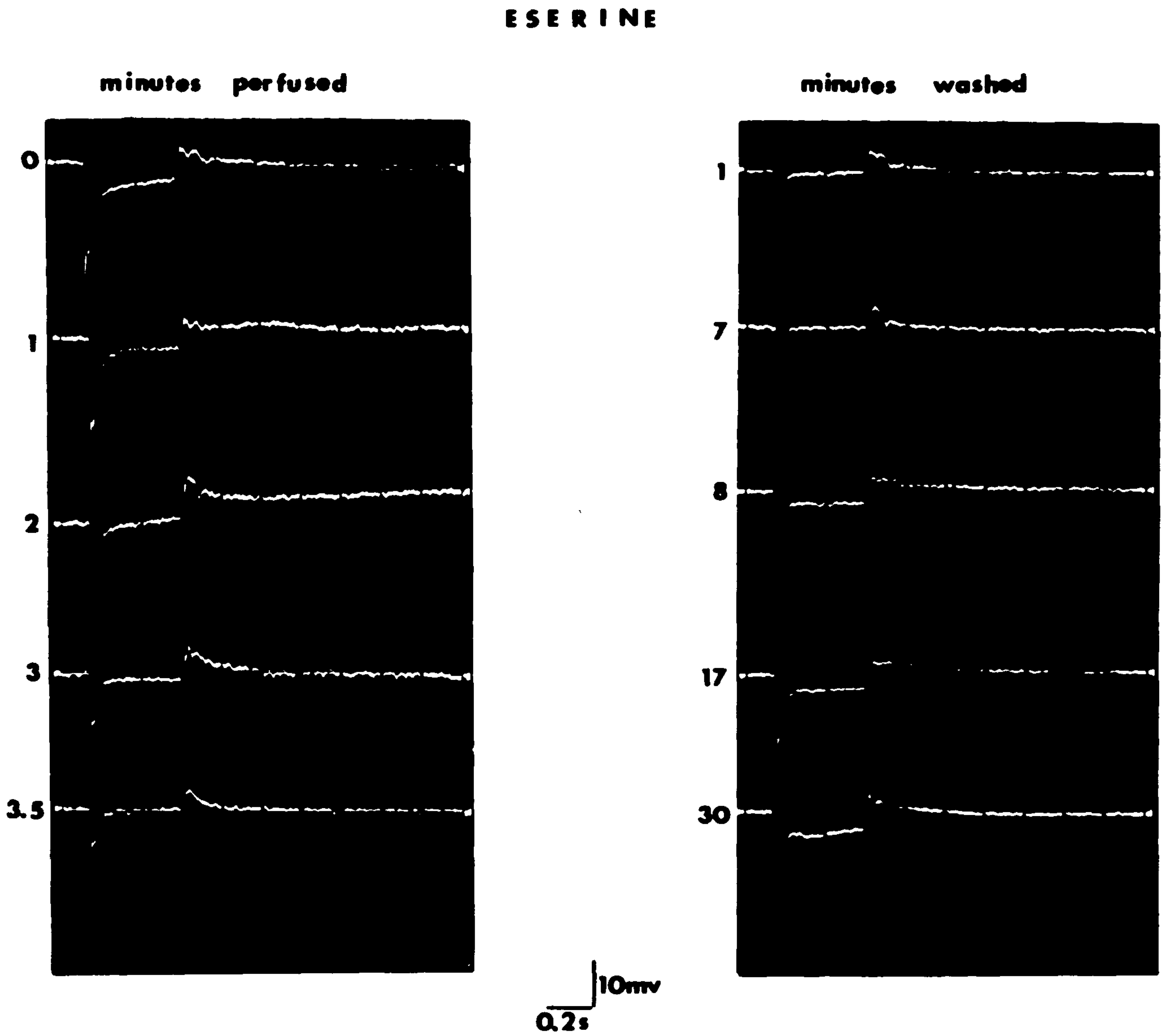


Figure 61

Figure 62

The L-neuron shown in the preceding figure (figure 61) was treated a second time with the same concentration of eserine (2 uM). The first response (upper left) was recorded after the preparation had been washing 32 minutes following the first eserine perfusion. Eserine again reduced the light response and caused the dark potential to depolarize about 8 mv. The dark potential remained about 1-2 mv more positive after 44 minutes of washing and the depolarizing OFF response was larger and more sustained. A third application of eserine to this cell (not shown) caused similar changes but the OFF response did not recover.

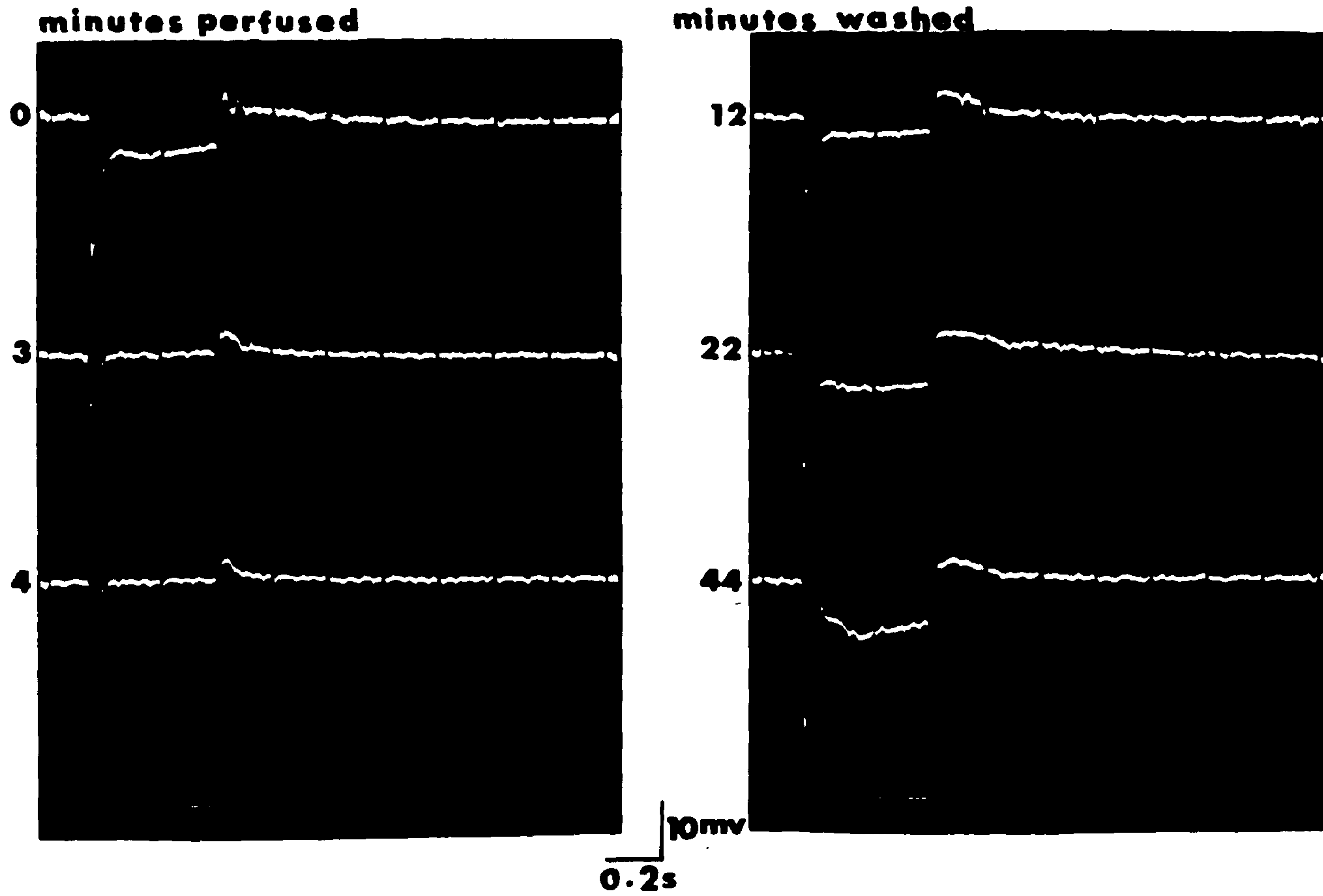


Figure 62

Figure 63

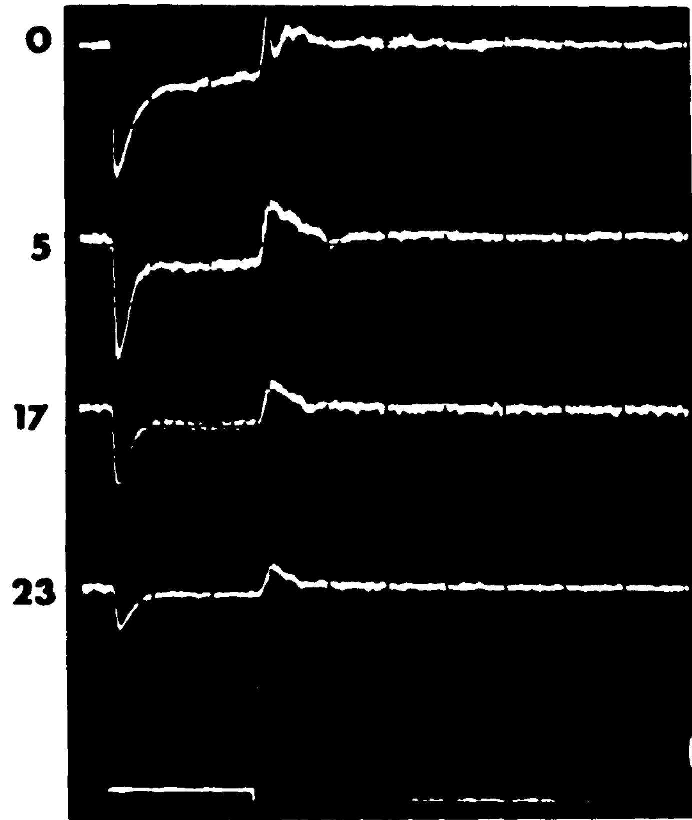
Effect of 10 mM carbachol on L-neuron response from intact nerve preparation. Each record shows superimposed responses to two consecutive test flashes. The change in the L-neuron response was similar to that observed with eserine (see figures 61, 62). There was a transient slowing and enhancement of the OFF response and a reduction in the magnitude of the light evoked hyperpolarization. Although the OFF response subsequently appeared smaller, it was not reduced to the same extent as the ON-transient and sustained components of the light response. After 23 minutes of perfusion the dark potential had depolarized 11 mv, compared to the potential before the application of carbachol. After 30 minutes of washing in Ringer the response waveform recovered except that the OFF response did not regain its oscillatory character. At the end of the wash period the resting potential was still 5 mv more positive than before carbachol. The data in this figure is represented in Graphs XXX-XXXVI.

Stimulus intensity: Log I = -3.

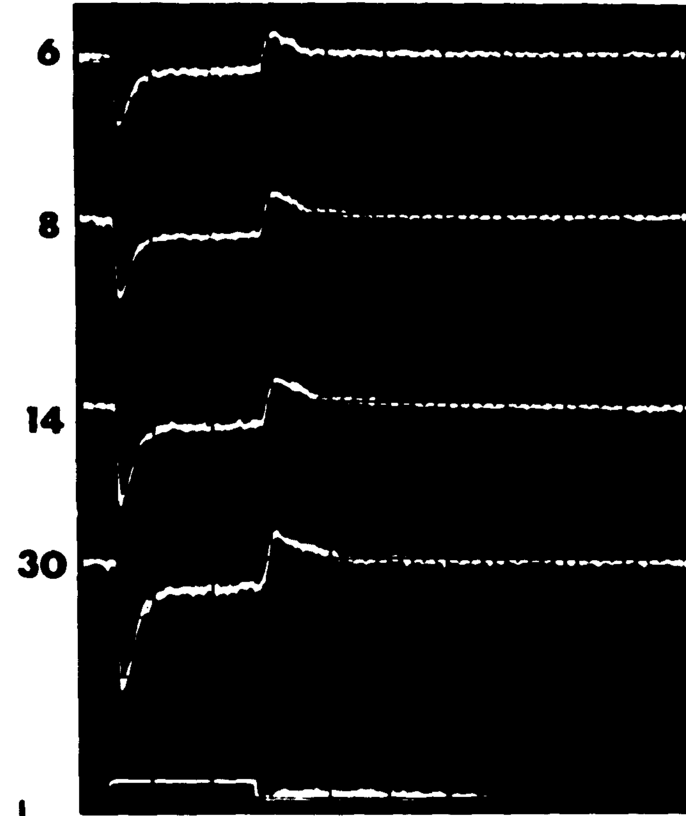
Resting potential (before carbachol): -59 mv.

CARBACHOL

minutes perfused



minutes washed



10mv
0.2s

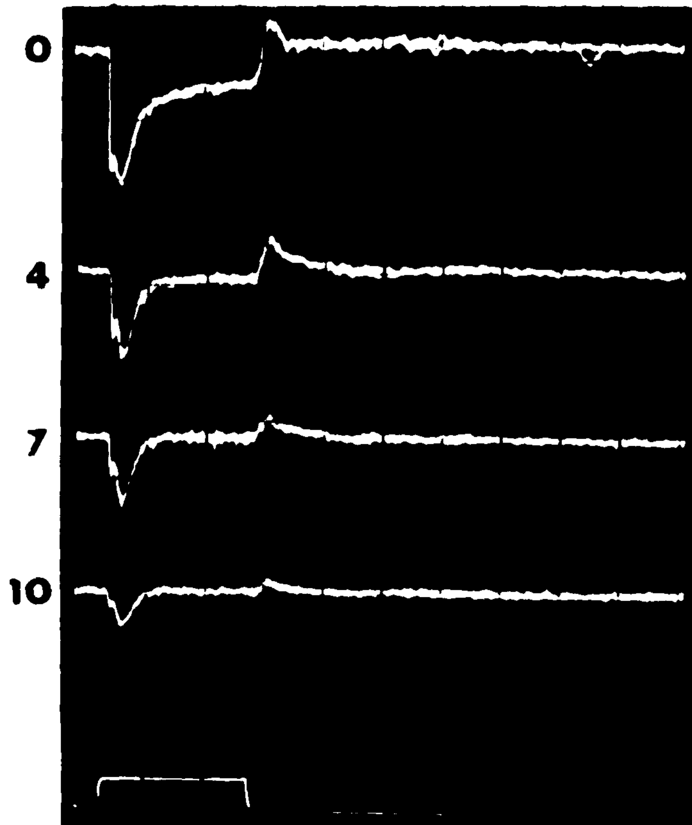
Figure 63

Figure 64

Effect of 10 mM carbachol on the L-neuron response from an intact nerve preparation. This is the same experiment shown in the preceding figure (figure 63) but these responses were recorded from a different cell. The cell shown here was impaled after the preparation had been washing 2 hours following the earlier carbachol perfusion described in figure 63. Each record shows superimposed responses to two consecutive test flashes. During carbachol perfusion there was a transient slowing and enhancement of the OFF response and a concurrent reduction in the magnitude of the light response. As perfusion continued all components of the L-neuron response became smaller. The waveform recovered after washing for 53 minutes. After 7 minutes perfusion the dark potential depolarized 4 mv (compared to before carbachol), but recovered after washing for 6 minutes. Note that the response after 6 minutes of washing is much smaller than before drug treatment, even though the dark potential was apparently at the same level. This cell was subsequently observed during an additional carbachol perfusion (see figure 65). The data from this experiment is represented in Graphs XXXVII-XLIII.

Stimulus intensity: Log I = -3.

CARBACHOL



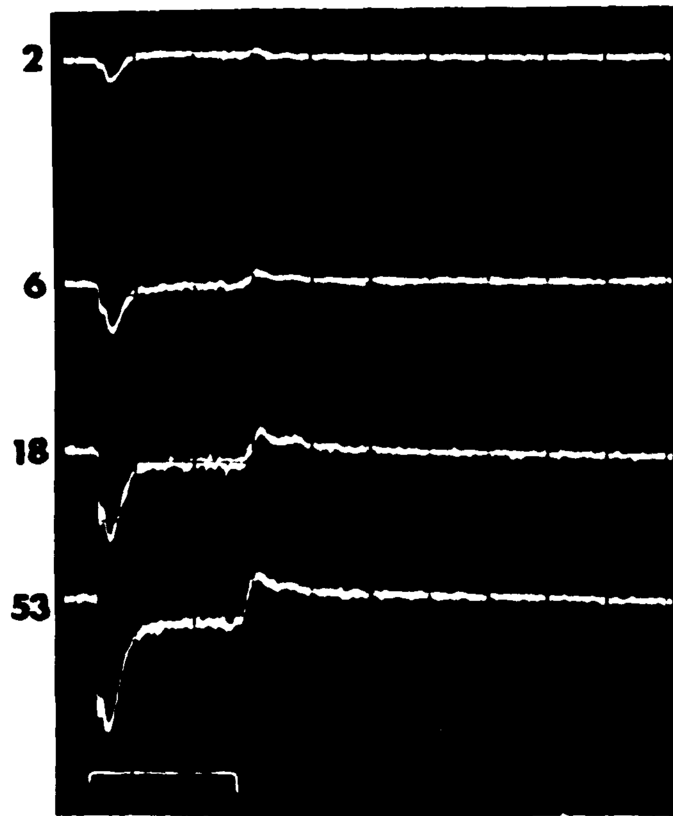


Figure 64

Figure 65

Effect of second carbachol perfusion (10 mM) on L-neuron response shown in preceding figure (figure 64). This is the third application of carbachol to this preparation. The effect of the first perfusion is shown in figure 61. Each record shows superimposed responses to two consecutive test flashes. The first record shown here (upper left) was taken after the preparation had been washing 74 minutes following the application of carbachol shown in the preceding figure (figure 64). The response waveform changed as described for the two earlier carbachol perfusions. However, the sustained portion of the light evoked hyperpolarization and the OFF response did not completely recover after washing 73 minutes. After 2 minutes of carbachol treatment, the dark potential depolarized 5 mv and remained at that level for the duration of the experiment. The data from this experiment is represented in Graphs XXXVII-XLIII. The resting potential was -40 mv when the electrode was withdrawn from this cell.
Stimulus intensity: Log I = -3.

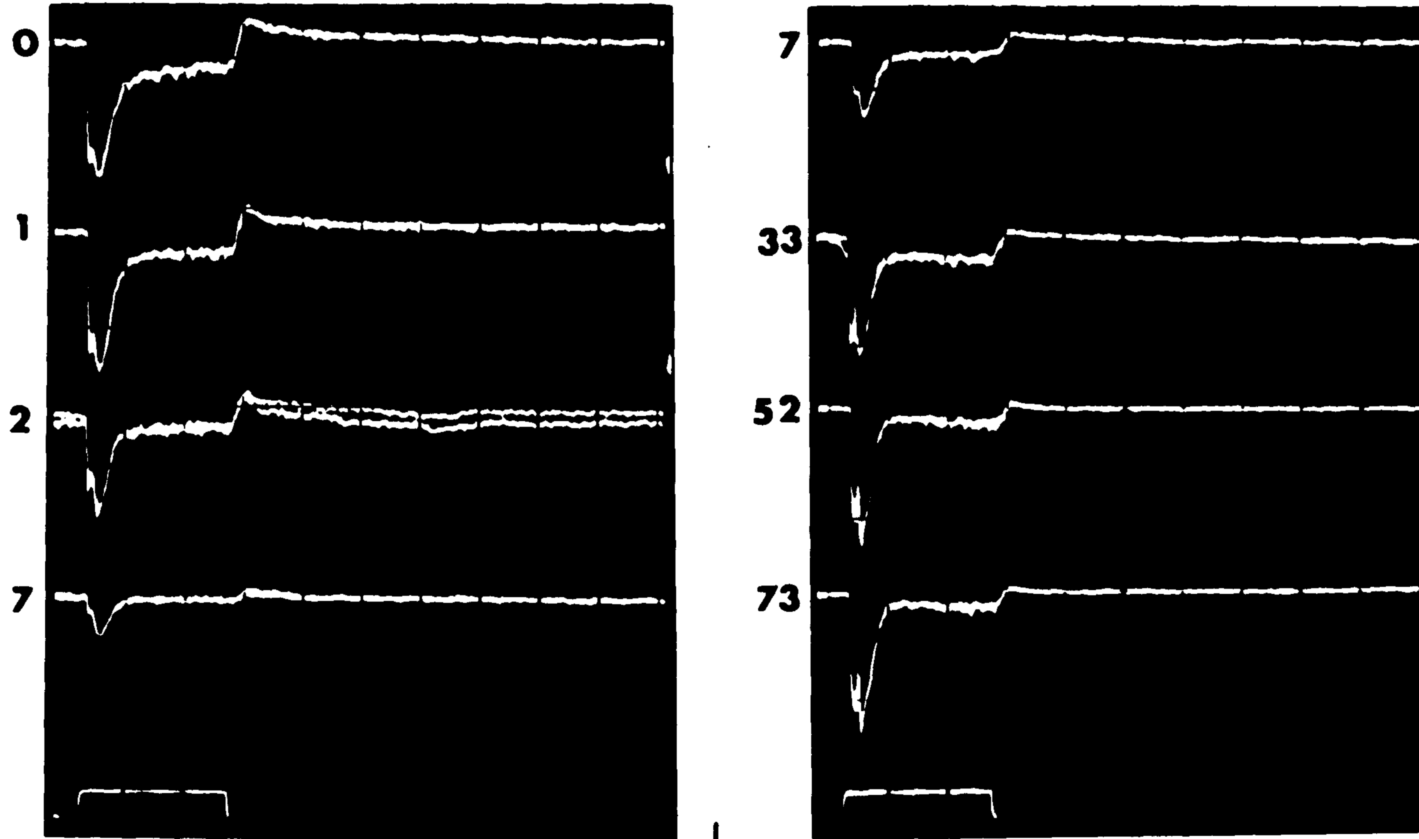


Figure 65

Figure 66

Effect of two concentrations of picrotoxin on the photoreceptor response from an intact nerve preparation. Each record shows superimposed responses to 5 or 6 consecutive test flashes. These same responses are shown at a slower sweep speed in the following figure (figure 67). During perfusion with the lower concentration of picrotoxin (0.16 mM) the response changed only a bit, but the potential between test flashes became less stable. This effect was reversed with washing. During the application of the more concentrated picrotoxin solution (1.6 mM) the OFF response gradually increased in amplitude and duration and the interflash potential became bistable. Washing was begun after 16 minutes perfusion with the higher dose, when the potential at light OFF had reached a hyperpolarized steady state. After a prolonged wash the response recovered almost completely to its former state. In this experiment, as in all others, the level of the light evoked depolarization was invariant, only the dark potential between test flashes was modified. The data from this experiment is represented in Graph XLIV, XLV, and XLVI.
Stimulus intensity: Log I = -2.
Resting potential (before picrotoxin): -50 mv.

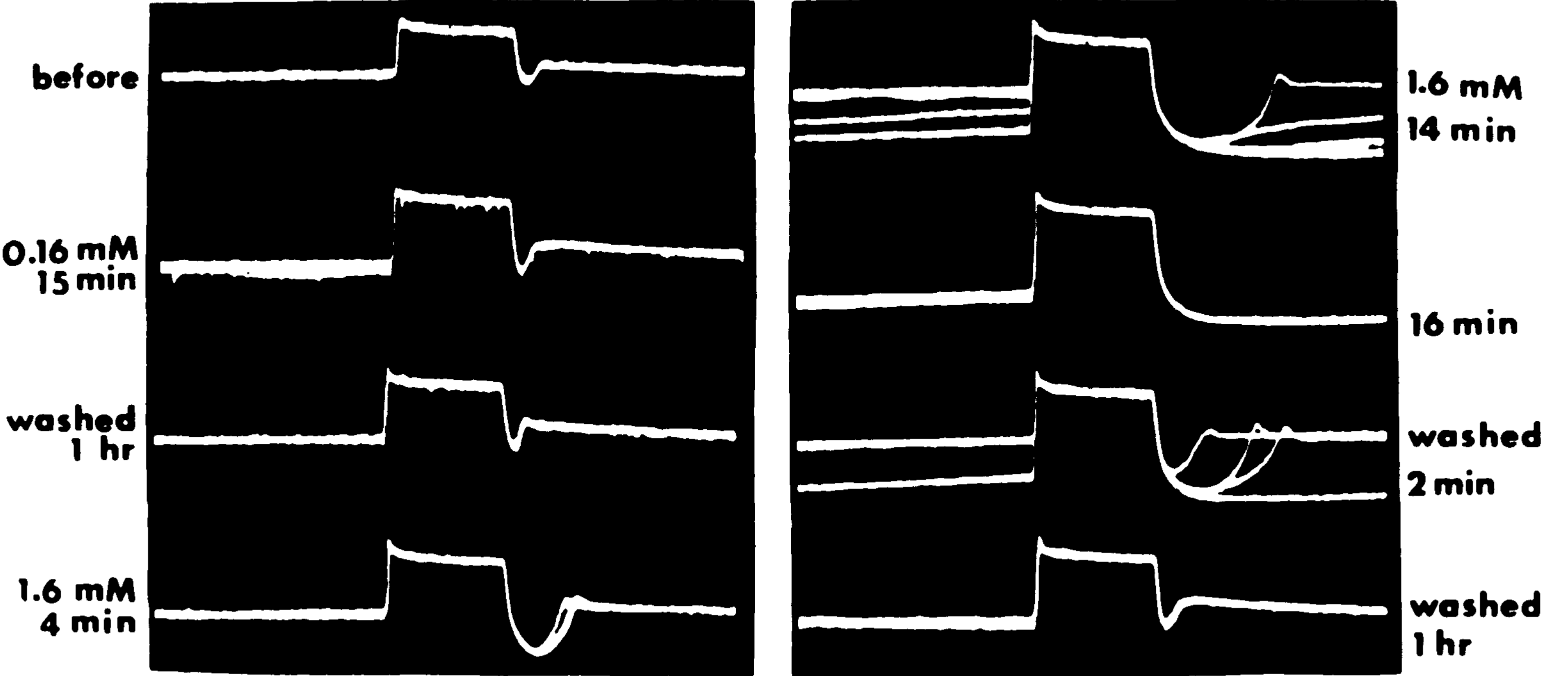
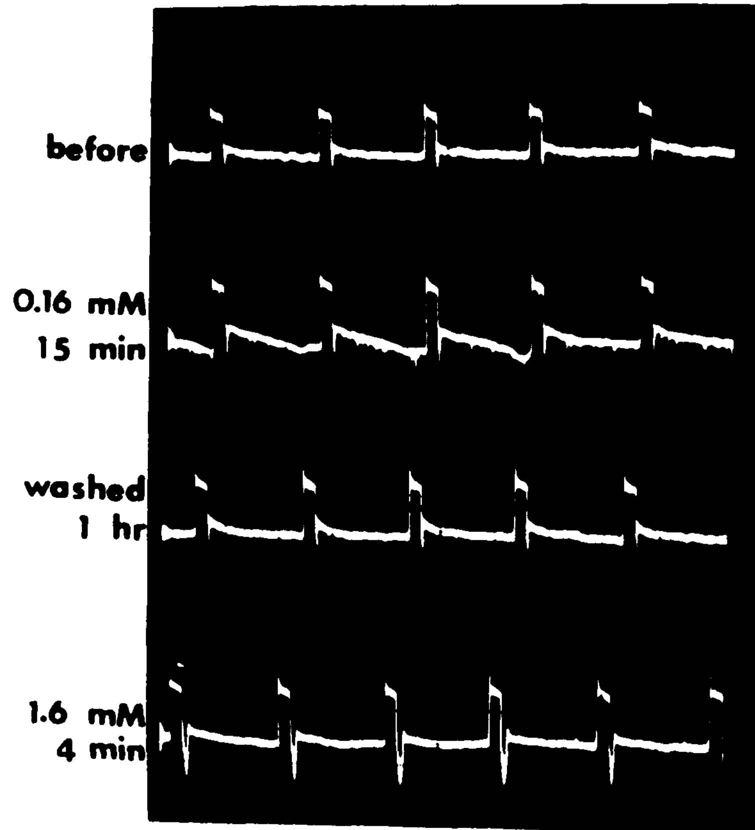


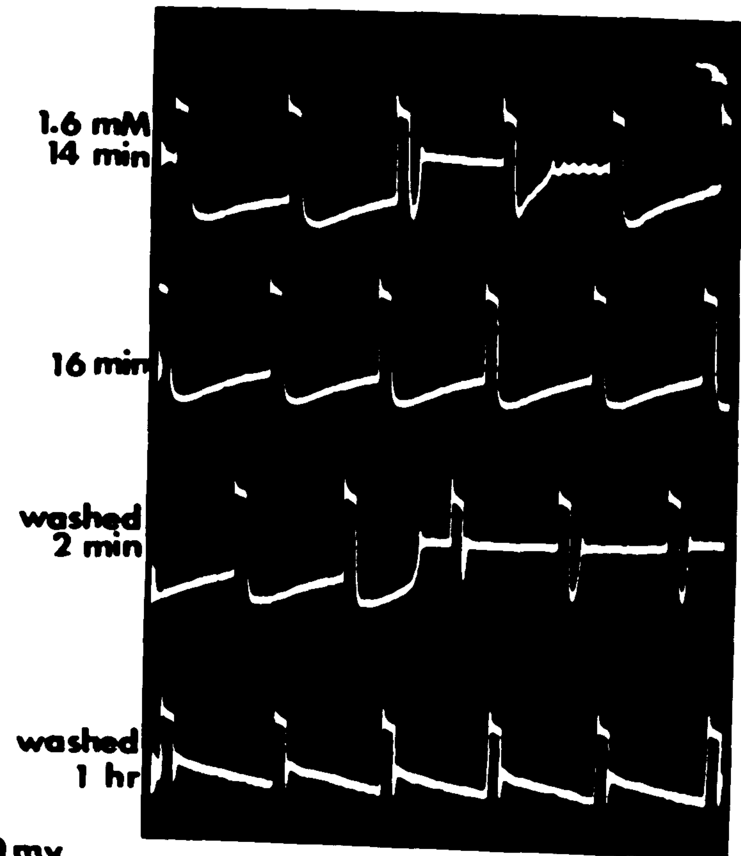
Figure 66

Figure 67

Effect of two concentrations of picrotoxin on the photoreceptor response from an intact nerve preparation. These are the same responses shown in the preceding figure (figure 66) except the reoords were photographed at a slower sweep speed (2 seconds per division). The data from this experiment is represented in Graphs XLIV, XLV, and XLVI.

PICROTOXIN





10 mv
2 s

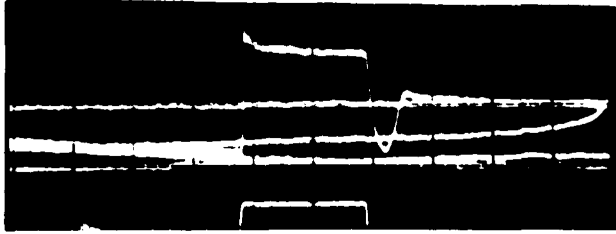
Figure 67

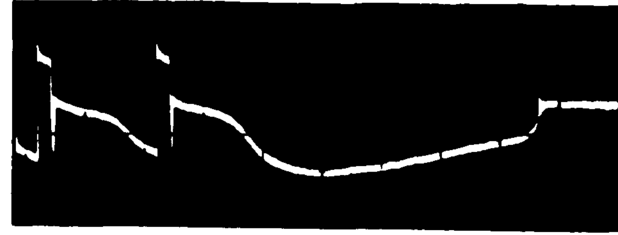
Figure 68

These responses were recorded from the same experiment shown in figures 66 and 67, recorded while the preparation was washing after the application of 1.6 mM picrotoxin. Top: After 6 minutes of washing the OFF response had regained a more normal appearance but the interflash potential was bistable. When the preparation was placed in the dark there was a delayed, sustained hyperpolarization which decayed back towards the original dark potential. Recovery was preceded by a rapid repolarization and small overshoot. Bottom: After 30 minutes of washing, the OFF response had recovered and the bistable condition was less pronounced. When the preparation was placed in the dark a delayed hyperpolarization of shorter duration preceded rapid repolarization back toward the original dark potential. In some experiments the dark adapted potential persisted at a more negative level for minutes. Occasionally such hyperpolarized cells showed intermittent transient depolarizations (see figure 39, B).

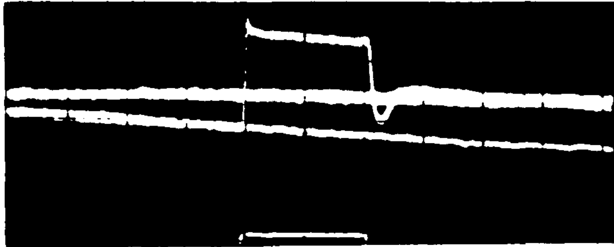
PICROTOXIN

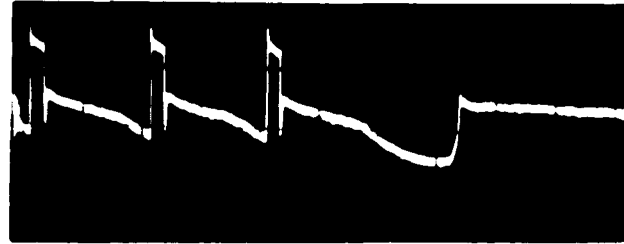
washed 6 min.





washed 30 min.





10mv
0.2s
2.0s

Figure 68

Figure 69

A post-illumination hyperpolarization (PIH) developed during the picrotoxin experiment shown in figures 66, 67, 68. The PIH, which appeared during the application of 0.16 mM picrotoxin, reversed during the first wash period. The PIH appearing during the application of the more concentrated solution of picrotoxin (1.6 mM) did not reverse (see figure 70).
Stimulus intensity: Log I = 0.

PICROTOXIN

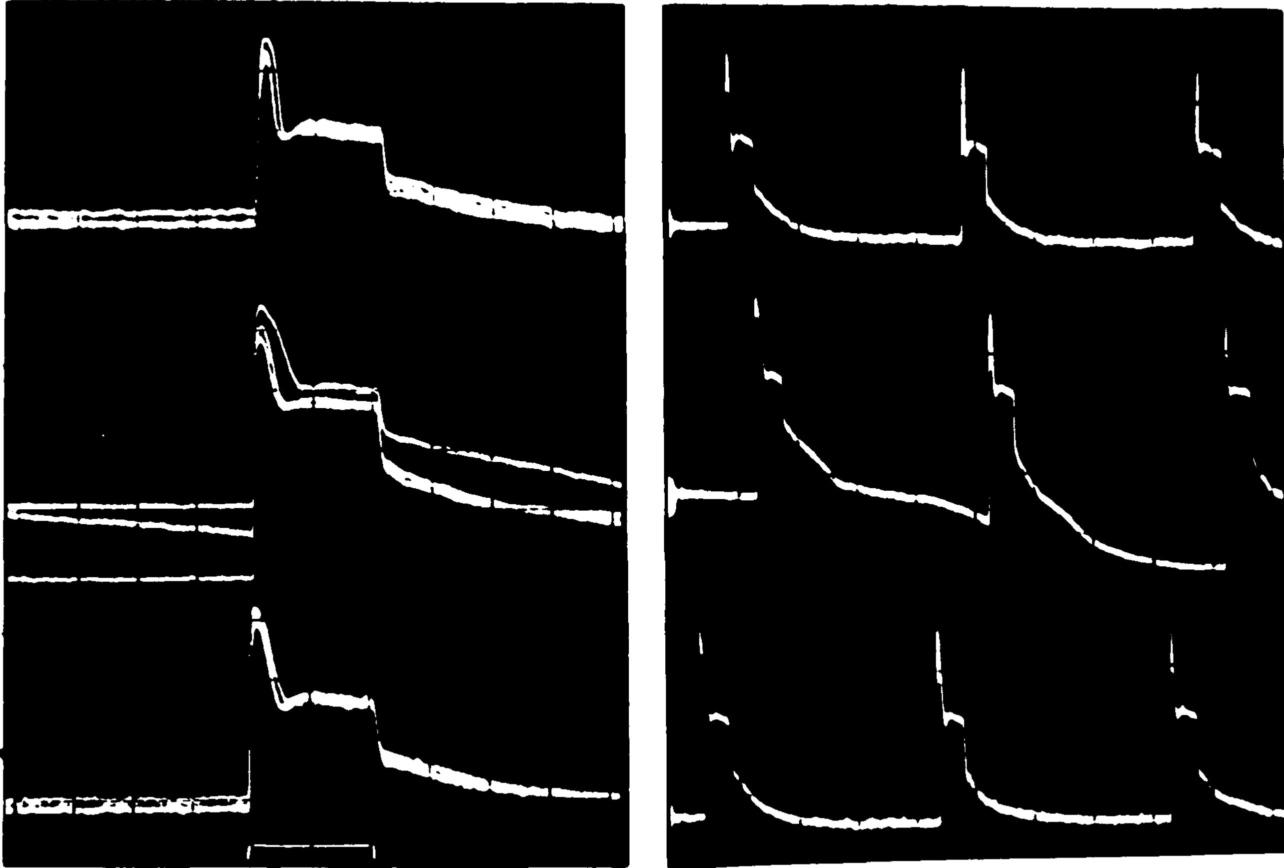


Figure 70

This figure is a continuation of the experiment shown in figures 66-69. A post-illumination hyperpolarization (PIH) developed during the application of picrotoxin. It reversed following exposure to the lower concentration (0.16 mM; see figure 69) but did not reverse following application of the more concentrated solution (1.6 mM) as shown here. This record shows 10 consecutive I = 0 test flashes presented to a dark adapted preparation. The level of the dark adapted potential is shown as a single sweep recorded before test flashing was initiated. The preparation was washing 4 minutes after exposure to 1.6 mM picrotoxin. The first response is the one showing the largest ON-transient and sustained depolarizing response to light. The first response of the dark adapted preparation also displayed a depolarizing after-potential at light OFF.

PICROTOXIN

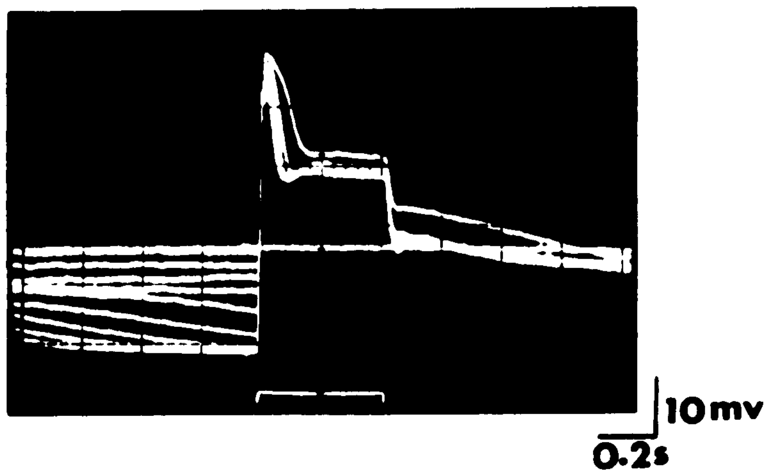


Figure 70

Figure 71

Effect of 1 mM picrotoxin on the photoreceptor response from a cut nerve preparation. Each record shows superimposed responses to 3 consecutive test flashes except for the record photographed during the initial wash period (upper right), which shows 5 superimposed responses. During perfusion the OFF response rapidly increased in amplitude and duration and oscillatory activity between test flashes was enhanced. During an equally brief wash period the OFF response decreased in duration and amplitude until the response appeared almost normal. The data in this experiment is represented in Graph XLVII.

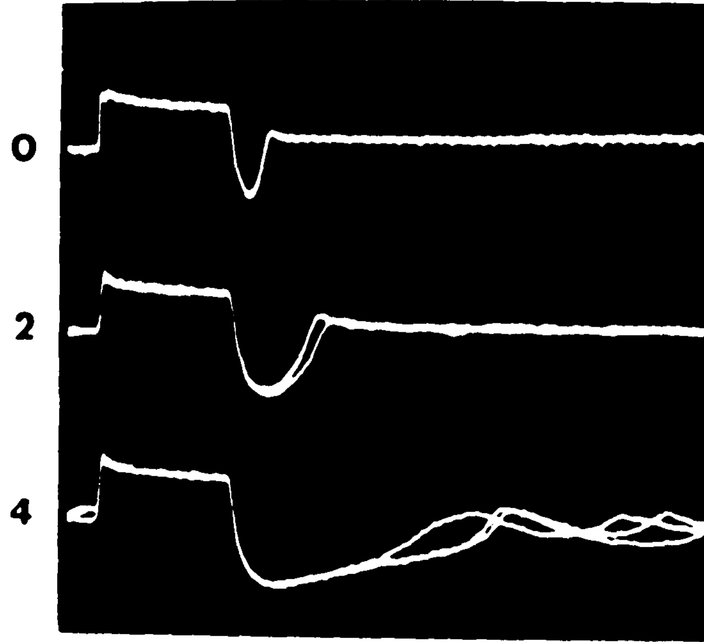
Stimulus intensity: Log I = -2.

Resting potential (before picrotoxin): -40 mv.

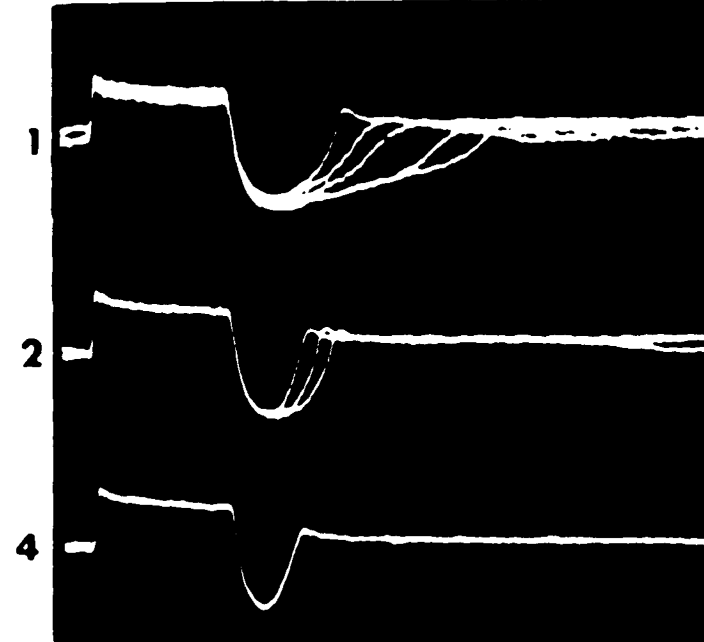
Figure 71

PICROTOXIN

minutes perfused



minutes washed



10 mv

0.2s

Figure 72

Picrotoxin (1.6 mM) enhanced the OFF response in an L-neuron from the same intact nerve preparation shown in figures 66 and 67. When this cell was impaled the preparation had been washing 2.5 hours after prior exposure to picrotoxin. During the wash period (before and after picrotoxin application shown here) slow depolarizing oscillations were seen between flashes. (Similar oscillations were also seen after exposure to cholinergic agents.) The cell depolarized during the wash period and did not recover its original waveform. The OFF response following less intense stimuli (Log I = 4 and Log I = -3) was also enhanced. All responses on the slow trace are superimposed on the expanded time scale.

Stimulus intensity: Log I = -2.

Resting potential (when impaled): -52 mv.

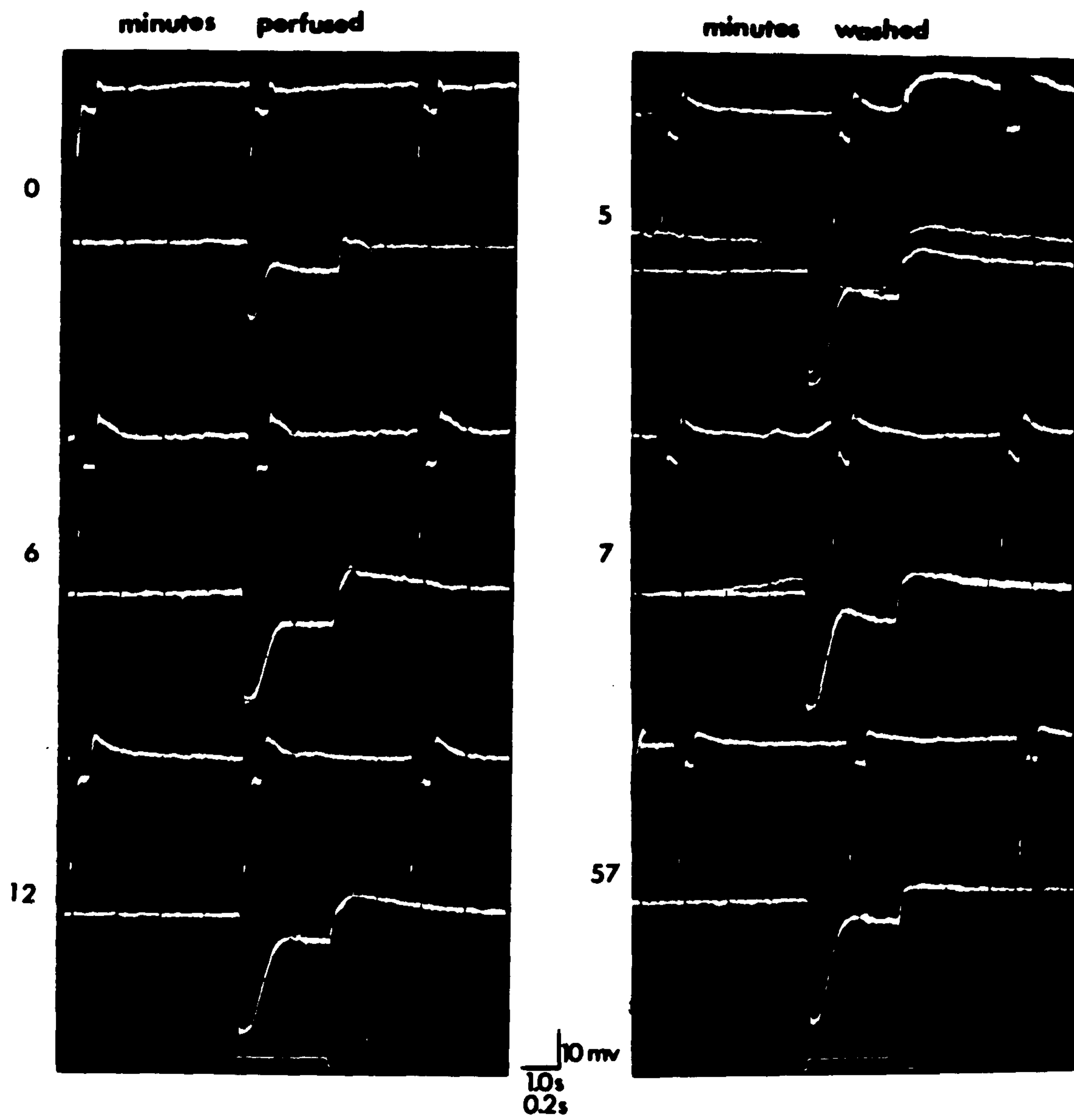


Figure 72

Figure 73

Effect of 6 mM cobalt chloride on the photoreceptor response from an intact nerve preparation. Initially, the OFF response gradually increased in duration. Following several bright test flashes the interflash potential became bistable and then hyperpolarized to a stable level after 30 minutes of exposure to cobalt. During the wash period the dark potential continued to hyperpolarize and the response did not recover its original waveform. The lower record shows superimposed responses before cobalt perfusion and after the preparation had been washing for 15 minutes. Earlier in the same experiment a different photoreceptor was perfused with the same concentration of cobalt for one hour, but only slight changes in the waveform of the light response were observed. The cell shown here was impaled after the preparation had been washing 1.5 hours following the earlier cobalt perfusion.

Stimulus intensity: Log I = -2.

Resting potential (before perfusion): -50 mv.

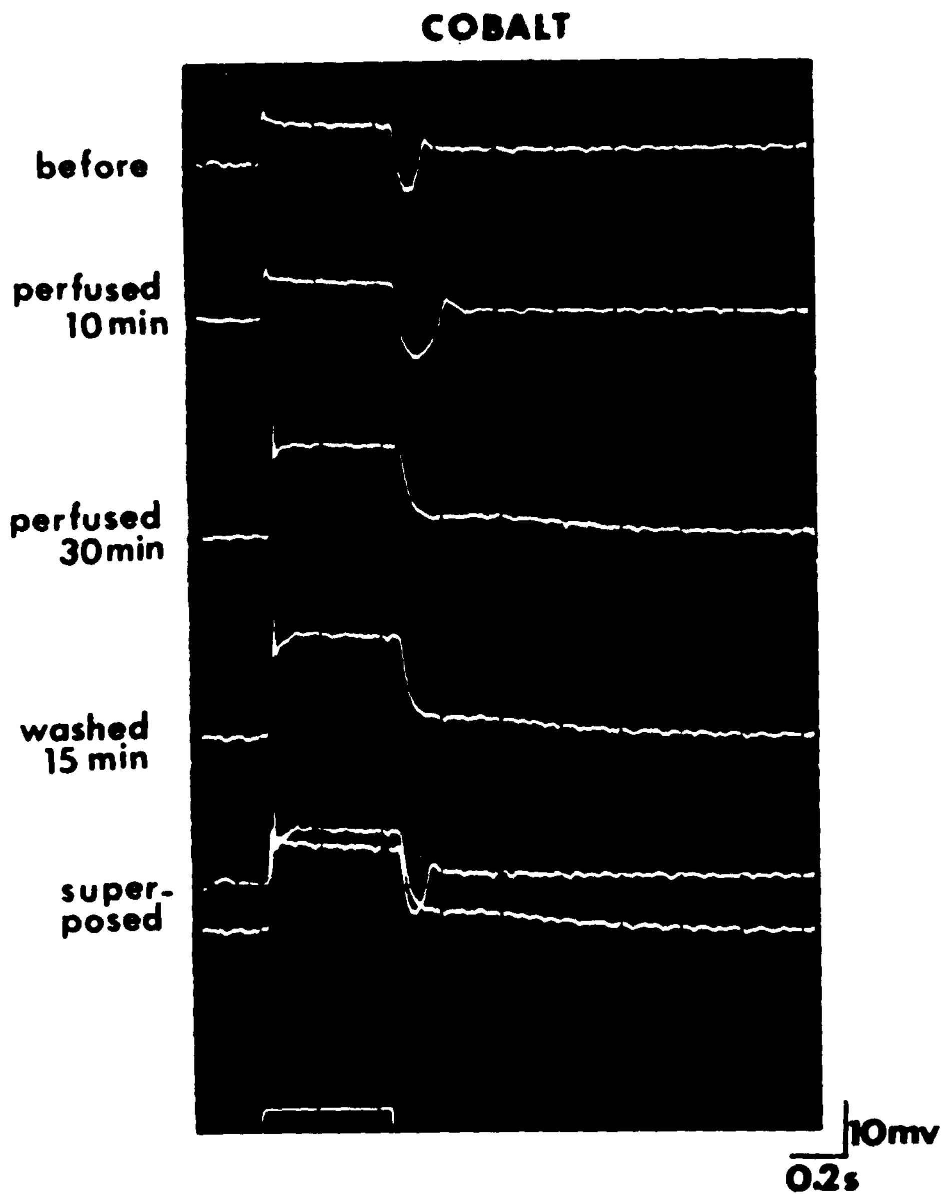


Figure 73

Figure 74

Effect of 4 mM cobalt chloride on an L-neuron response from an intact nerve preparation. (This cell was impaled after the preparation had been washing 2 hours following a 12-minute application of 4 mM cobalt.) Each record shows superimposed responses to 3 consecutive test flashes. During the application of cobalt the OFF response and sustained component of the light response were eliminated while the amplitude of the ON-transient became larger. (After 10 minutes perfusion the dark potential had depolarized 8 mv.) Partial recovery of the original waveform and dark potential was seen after a prolonged wash period. The change in waveform observed during the application of cobalt was very similar to that seen during curare perfusion (see figures 46 and 49).

Stimulus intensity: Log I = -2.

Resting potential (before cobalt): -48 mv.

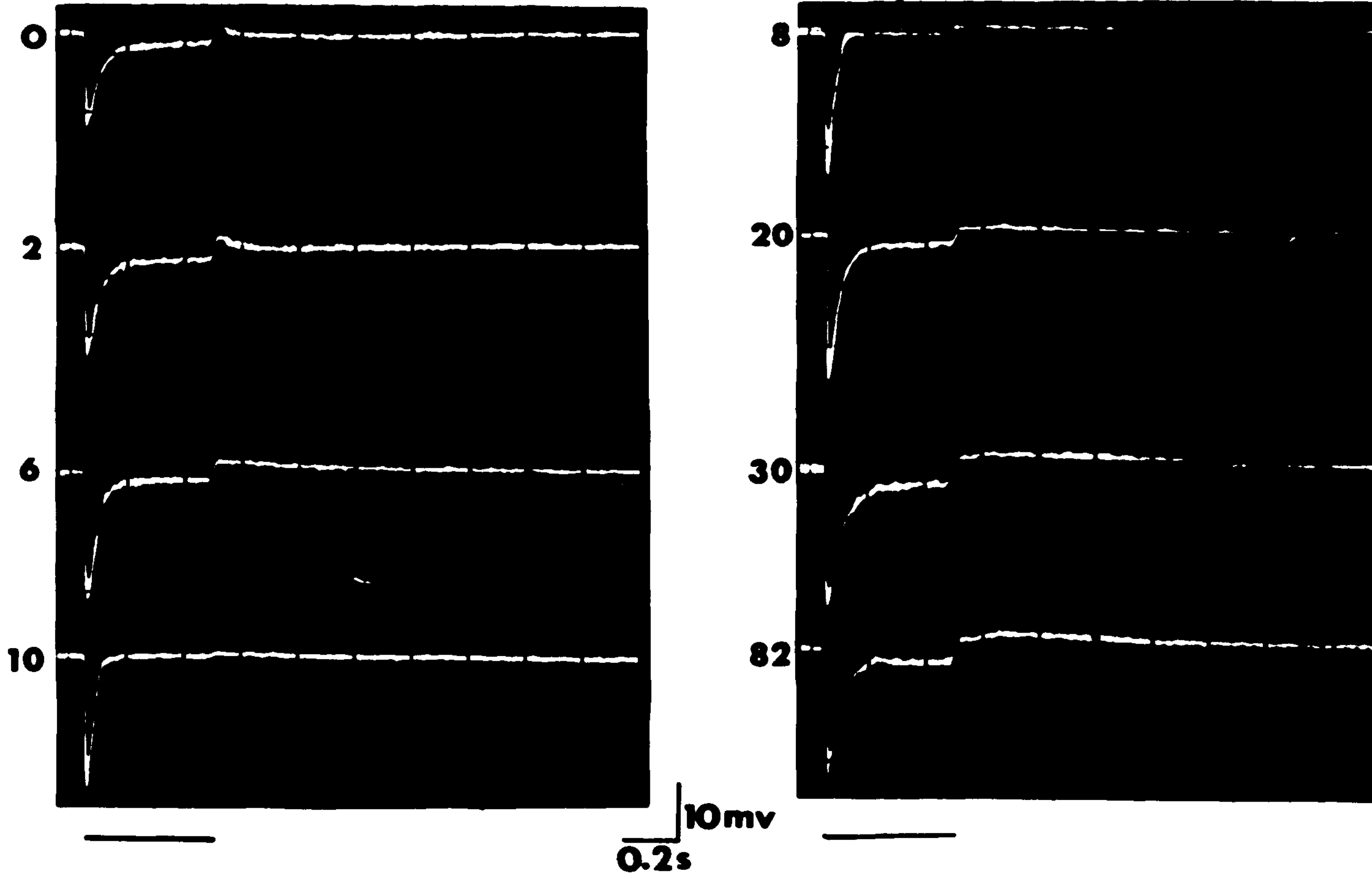
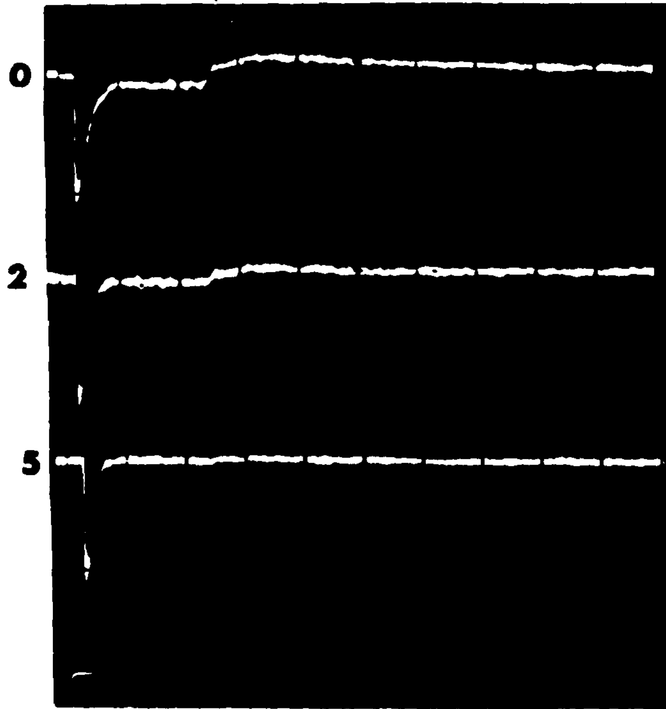


Figure 74

Figure 75

The cell shown in the preceding figure (figure 74) was perfused a second time with 4 mM cobalt. During the second cobalt application the light response was almost completely blocked. Each record shows superimposed responses to 3 consecutive test flashes. The second perfusion was initiated after the cell had been washing for 95 minutes. The dark potential depolarized 10 mv after 5 minutes perfusion and was 4 mv more positive after 12 minutes of washing.

COBALT



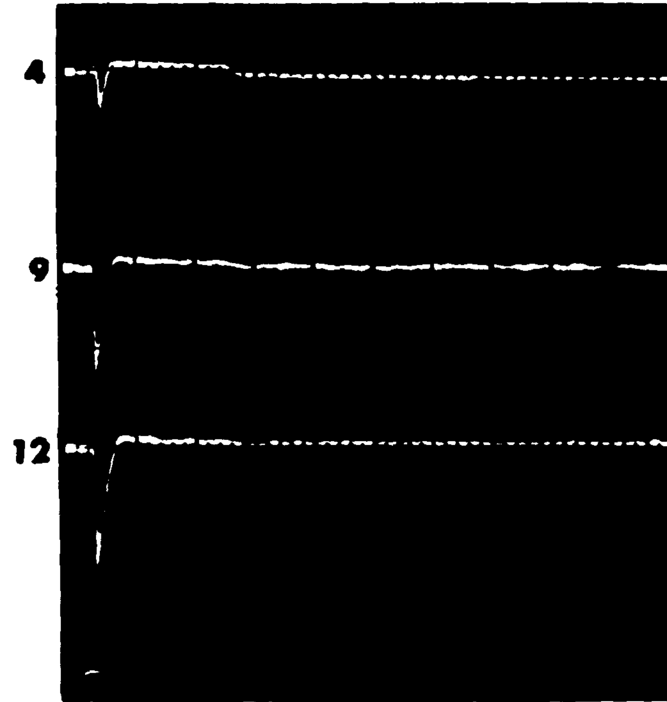


Figure 75

Figure 76

Effect of 4 mM cobalt chloride on a cut nerve photoreceptor showing enhanced oscillatory activity. All responses on the slow sweep to the right are shown superimposed on the faster sweep to the left. Cobalt reversibly blocked the spike-like components of the dark oscillations, but did not block all the large, slow hyperpolarizing oscillations. These spontaneous hyperpolarizations between test flashes are distinct from the OFF response. The hyperpolarizations shown above (after 10 minutes perfusion) increased to 15 mv in amplitude and 5 seconds in duration (not shown) before washing was initiated (after 19 minutes perfusion). Note that cobalt does not block the ON-spike, which is sensitive to tetradotoxin (Chappell and Dowling, 1972). I noted that the latency of the ON-spike is apparently a function of the dark potential prior to light ON. The lower record shows 3 superimposed responses stored over a period of 12 minutes, before and during the application of cobalt (1 response before cobalt, one response after 10 minutes perfusion, and one sweep in the dark after 10 minutes perfusion). After treatment with cobalt, the dark potential in this receptor reached an intermediate level in between the more positive and negative value observed in the more oscillatory condition. This more stable level of dark potential measured -50 mv.

Stimulus intensity: Log I = -2.

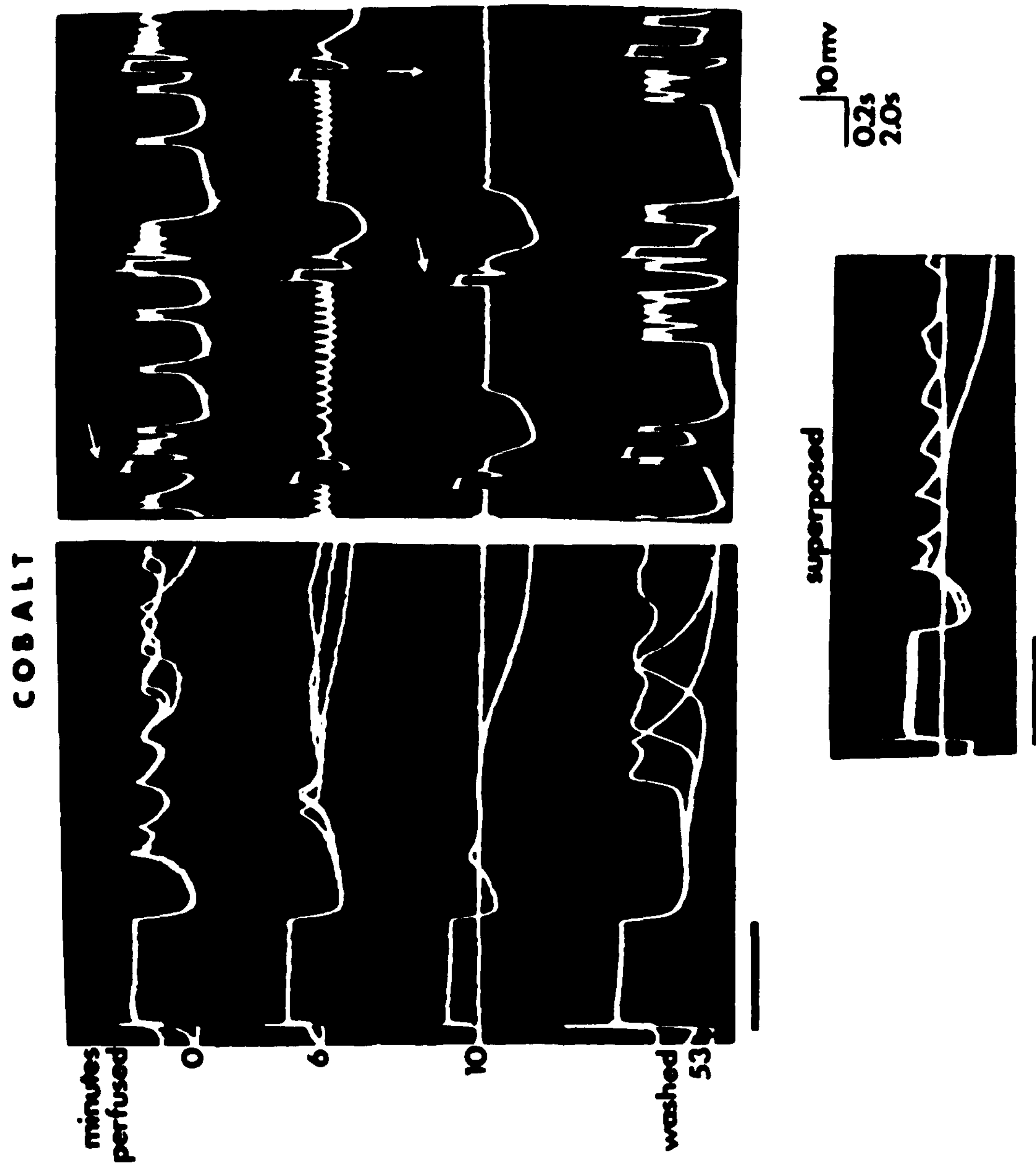


Figure 76

Figure 77

Effect of 4 mM cobalt on the dark oscillatory activity in a photoreceptor from a cut nerve preparation. These records were taken from the same experiment shown in the preceding figure (figure 76). Note slower sweep speed (5 sec/div). The large sustained hyperpolarization following the brightest test flash (Log I = 0) was invariably observed in cut nerve preparations, but not in animals with intact ocellar nerves before the application of drugs. In this experiment cobalt blocked the more rapid depolarizing oscillations but had little effect on the spontaneous hyperpolarizations.

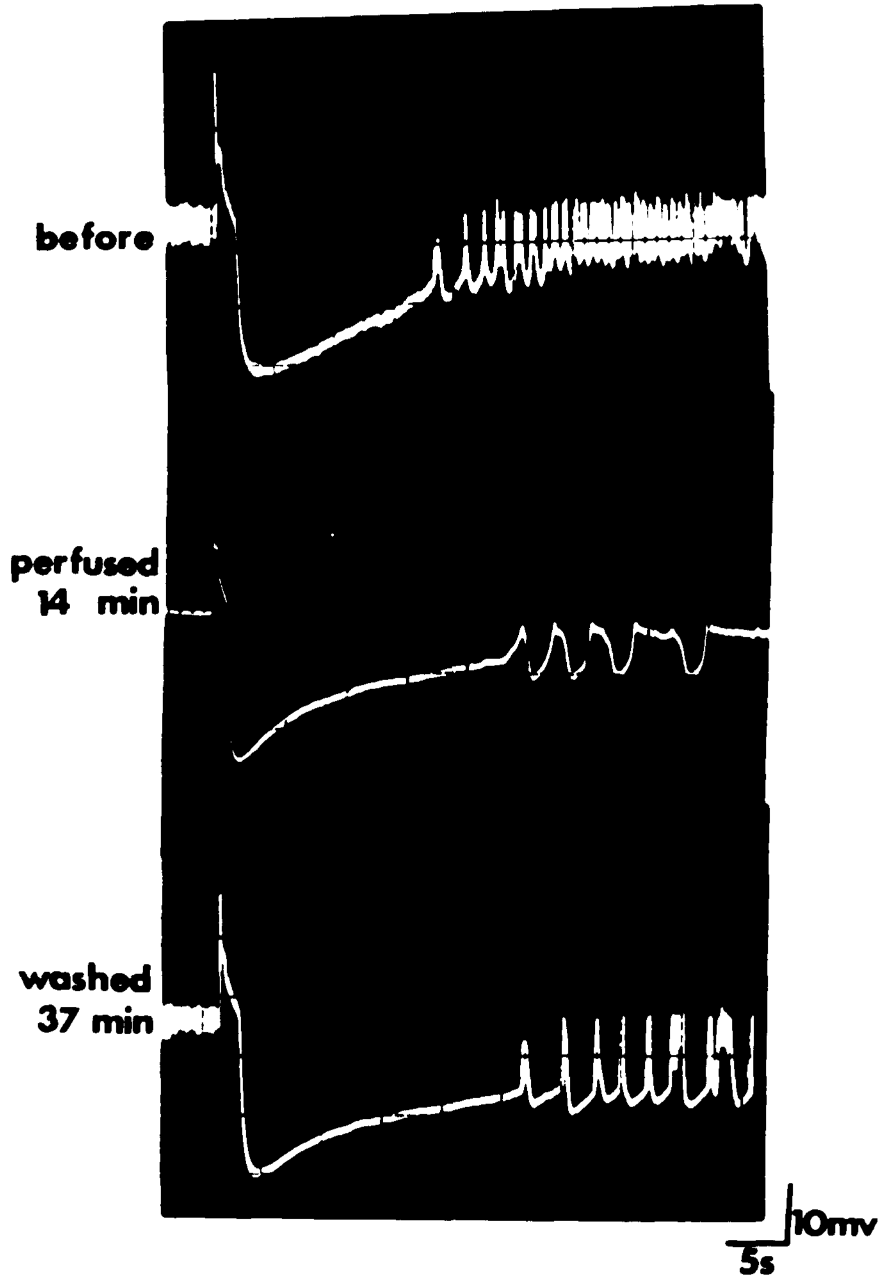


Figure 77

Figure 78

Effect of 4 mM cobalt on a photoreceptor response from a cut nerve preparation. In this experiment, the application of cobalt blocked, or delayed the onset of an unusually large, sustained hyperpolarization following light OFF. This preparation had previously been treated with cobalt, and had been left washing for more than two hours before the cell shown here was impaled. (The first cell studied was lost while the preparation was unattended. That cell had a normal appearing OFF response and displayed a moderate oscillatory activity between test flashes.) The cell shown here was observed during two applications of cobalt (both 4 mM). Initially a depolarizing notch appeared on the falling phase of the sustained OFF response. This depolarizing notch seemed to increase in duration until the OFF hyperpolarization was blocked, leaving a small residual "normal" appearing OFF response (see "washed 6 min"). It was not possible to determine if the sustained hyperpolarization was actually blocked, or if its onset was merely delayed for a longer period of time than the interval between test flashes. This experiment was carried out under continuous test flashing of constant intensity (Log I = -2) and frequency. These stimulus conditions were not changed during the 3 hours preceding the impalement of this cell.

After 6 minutes perfusion the potential between test flashes appeared stable and the light response remained constant. During

(continued...)

Figure 78 (continued)

the wash period the reverse sequence of changes occurred. A delayed hyperpolarization following light OFF appeared, and the latency of this hyperpolarization gradually decreased until it was seen as an unusually large OFF response (see "washed 19 min"). A similar effect was seen during the second application of cobalt to this cell, but this time either the depolarization did not persist as long or the hyperpolarization was not delayed as long. Therefore, the interflash potential took on a bistable appearance (see "washed 27 min").

COBALT

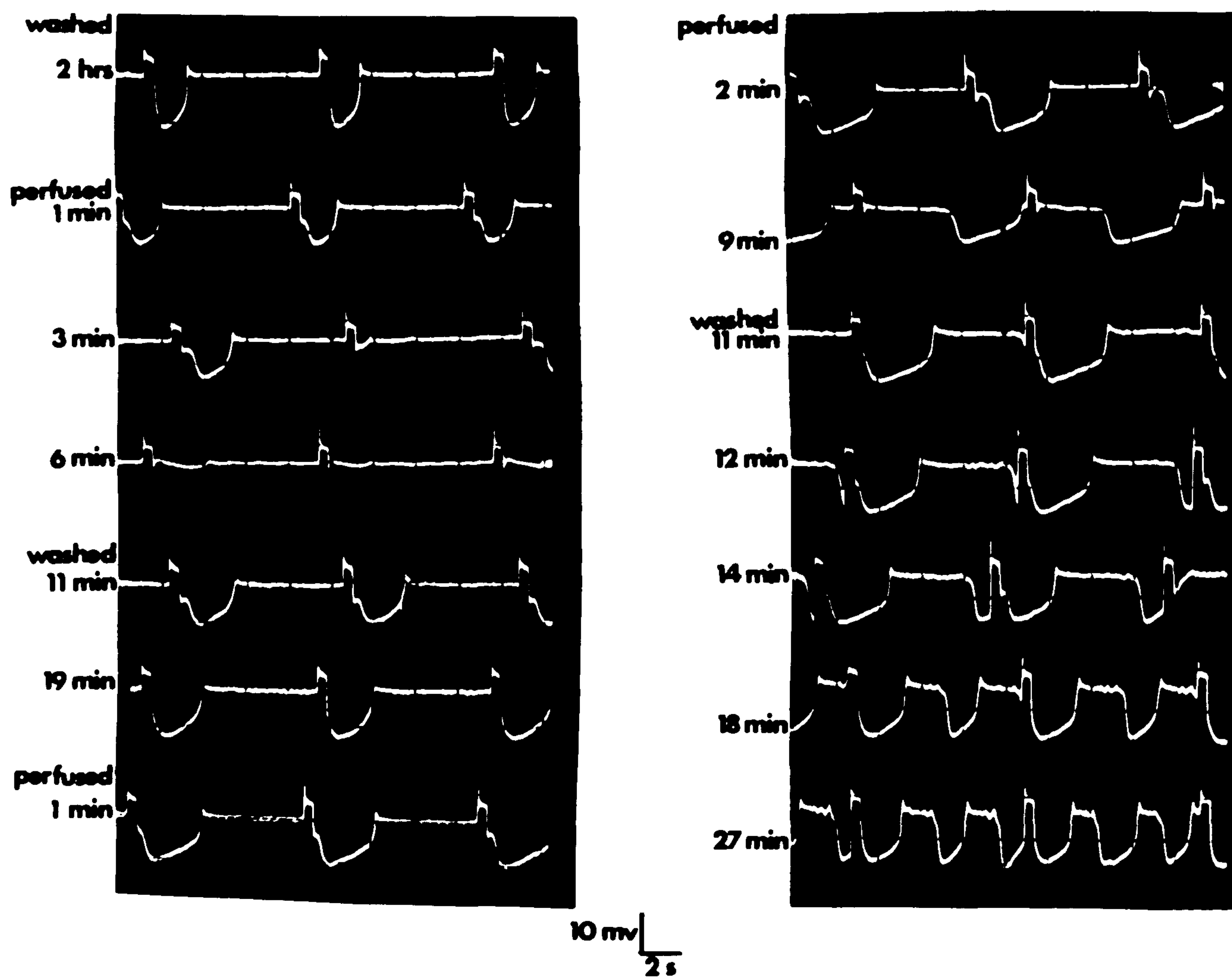


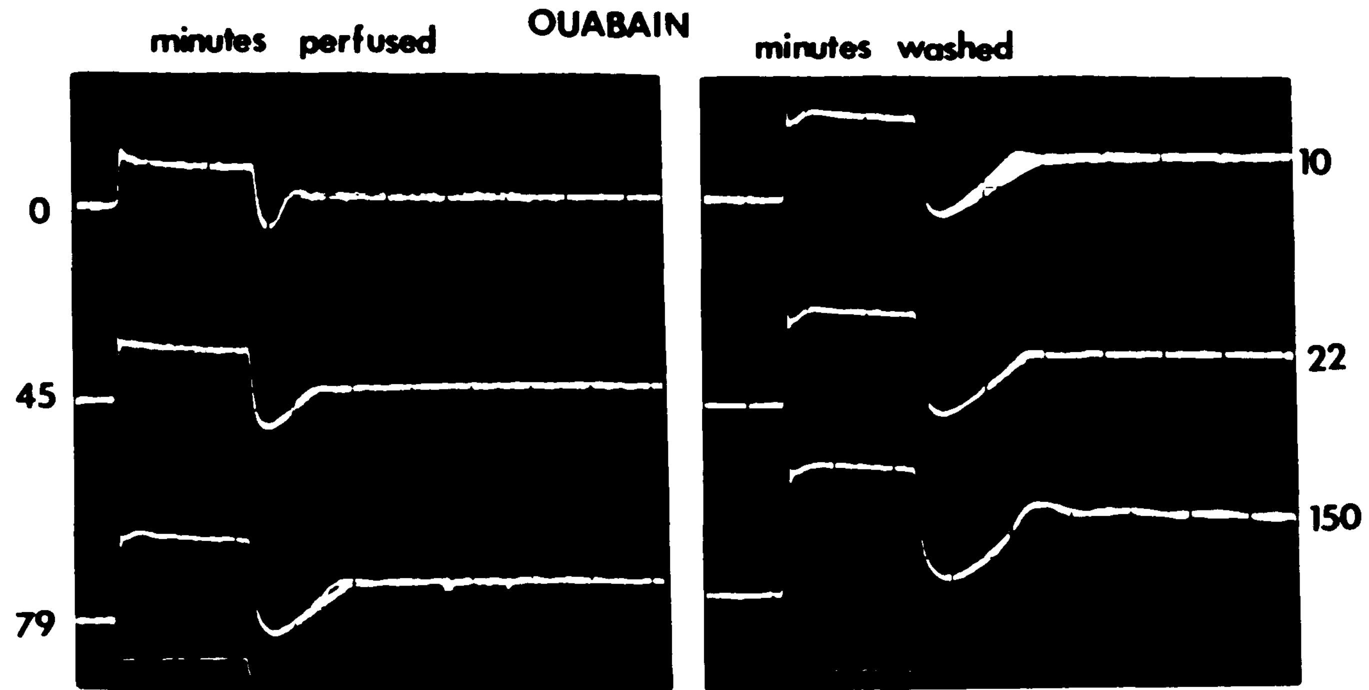
Figure 78

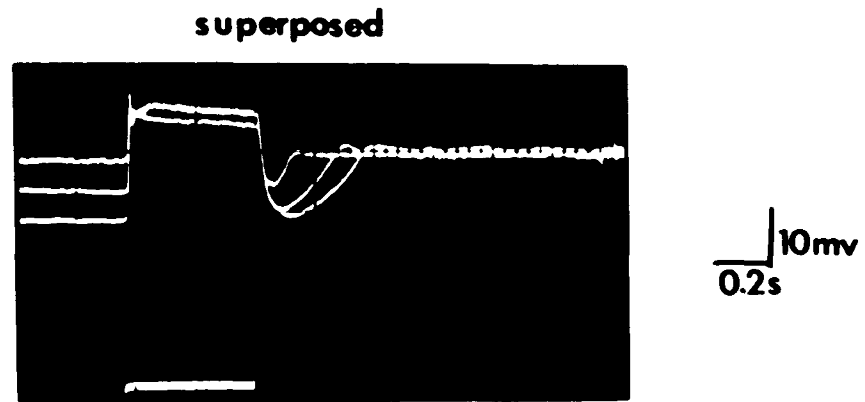
Figure 79

Effect of ouabain on photoreceptor response. This photoreceptor from an intact nerve preparation developed pronounced bistable behavior during prolonged exposure to 10 uM ouabain. Each record shows superimposed responses to 4 consecutive test flashes. These responses are shown as part of a single trace recorded at a slower sweep speed in figure 80. The superimposed record on the bottom of this figure shows 3 receptor responses stored over a period of 3.5 hours (before ouabain, perfused 103 minutes, washed 2.5 hours). The superimposed responses were recorded at the approximate times indicated above but are not necessarily the same responses. The changes in waveform shown here and in figure 80 are characteristic of the changes in waveform observed during the development of the bistable condition.

Stimulus intensity: Log I = -2.

Resting potential (before ouabain): -42 mv.





10mv
0.2s

Figure 79

345

Figure 80

Photoreceptor from intact nerve preparation developed pronounced bistable behavior during the application of 10 uM ouabain. These are the same responses shown in the preceding figure (figure 79) but are recorded at a slower sweep speed (2 sec per division).

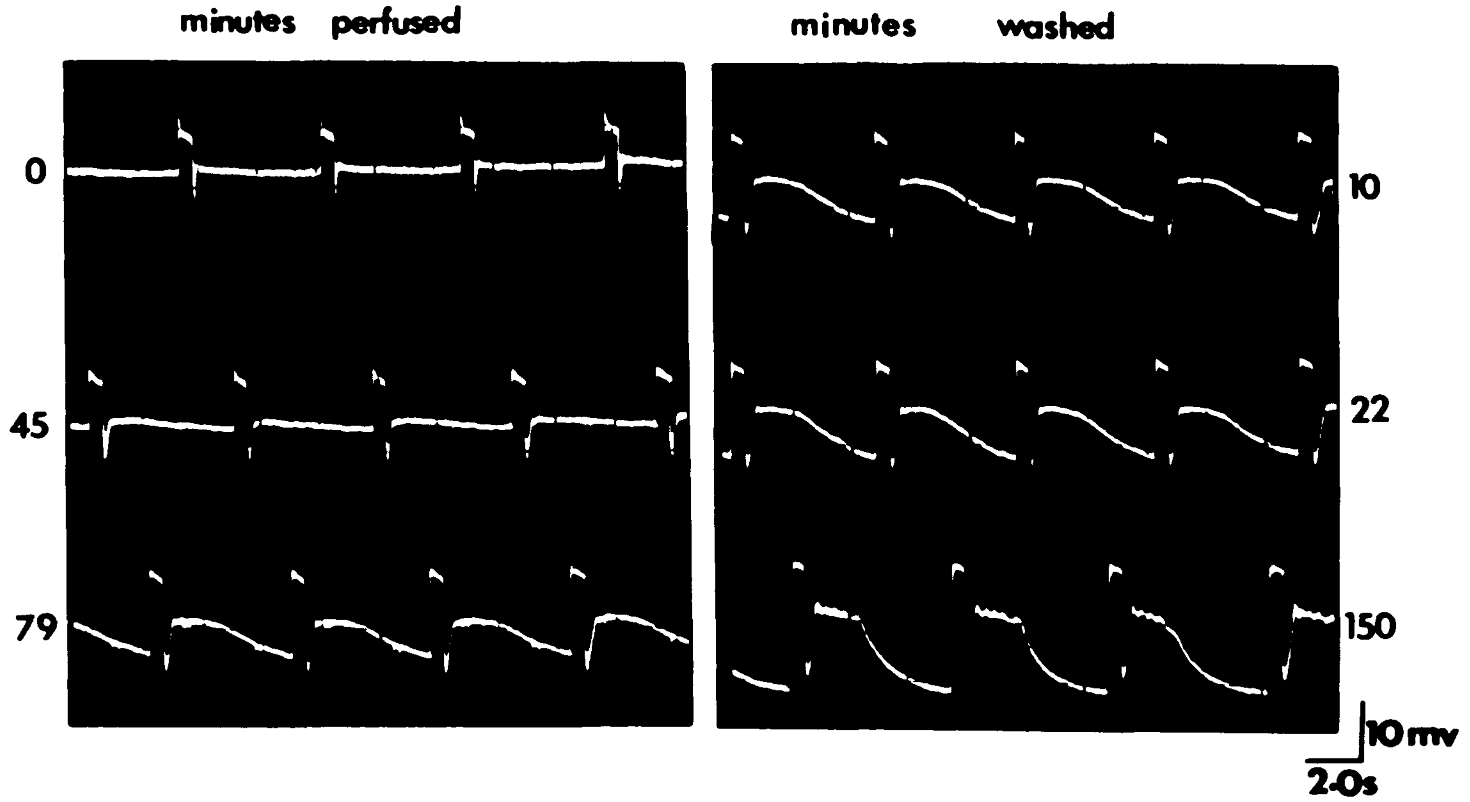


Figure 80

Figure 81

A post-illumination hyperpolarization developed in a photoreceptor from an intact nerve preparation during the application of ouabain. This is the same experiment shown in figures 79 and 80.

A: Before ouabain perfusion no post-illumination hyperpolarization was detected, which is normally the case for photoreceptors from intact nerve preparations. All the responses on the slow sweep (to the right) are superimposed on the faster time scale (to the left).

B: A post-illumination hyperpolarization in response to unattenuated (Log I = 0) test flashes was seen after 85 minutes perfusion with 10 uM ouabain. The cell recovered to a stable dark potential after approximately 25 second in the dark.

Stimulus intensity: Log I = 0

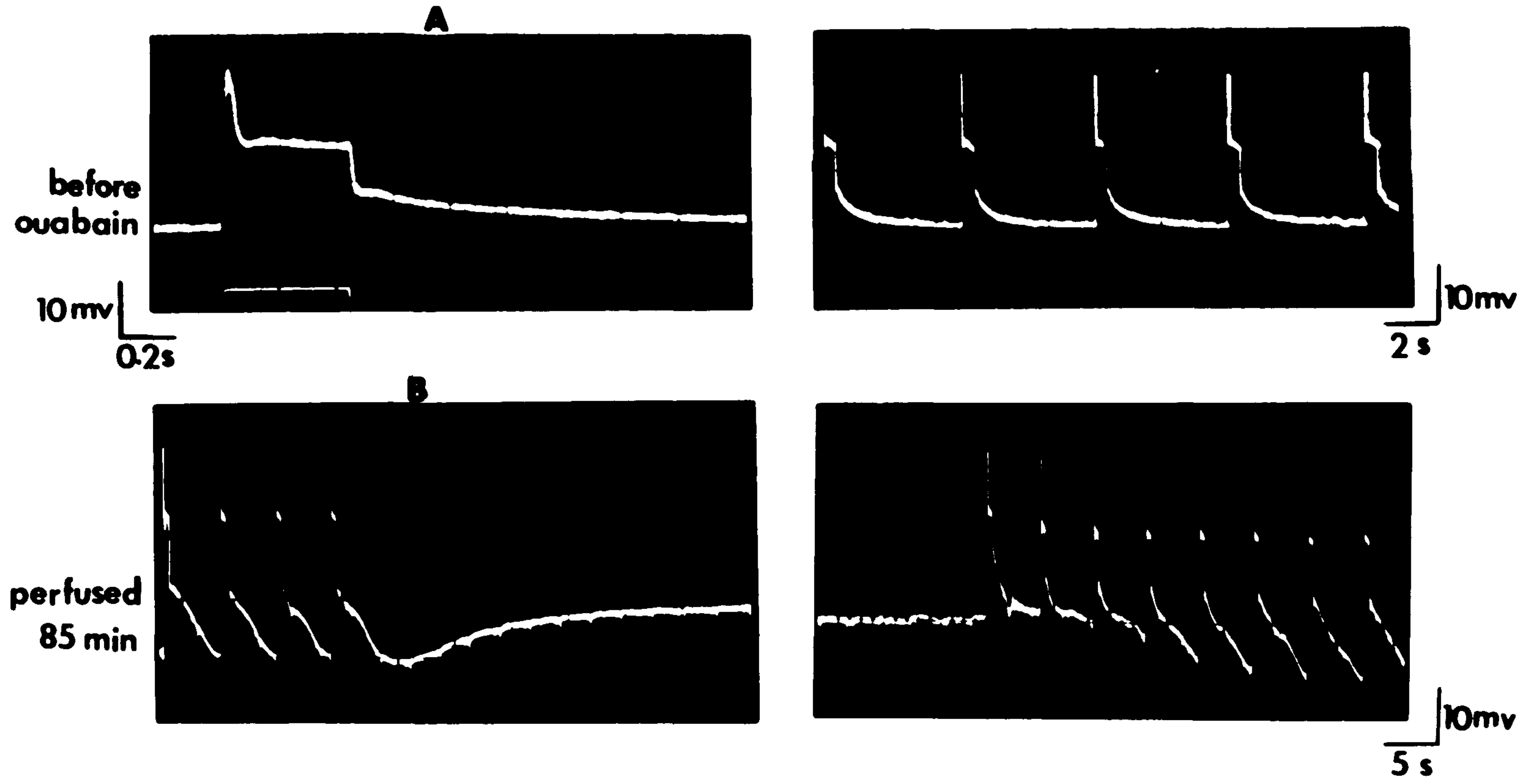


Figure 81

Figure 82

A post-illumination hyperpolarization developed in a photoreceptor from an intact nerve preparation (different experiment than in figure 81) during the application of 10 uM ouabain.

Top: Consecutive responses to Log I = 0 test flashes before ouabain perfusion. No post-illumination hyperpolarization was seen before the application of ouabain.

Middle: A post-illumination hyperpolarization developed after 15 minutes perfusion with 10 uM ouabain. The beginning of the trace shows the dark adapted potential.

Bottom: The amplitude of the post-illumination hyperpolarization gradually increased and reached a steady state after 1-2 minutes of continuous test flashing.

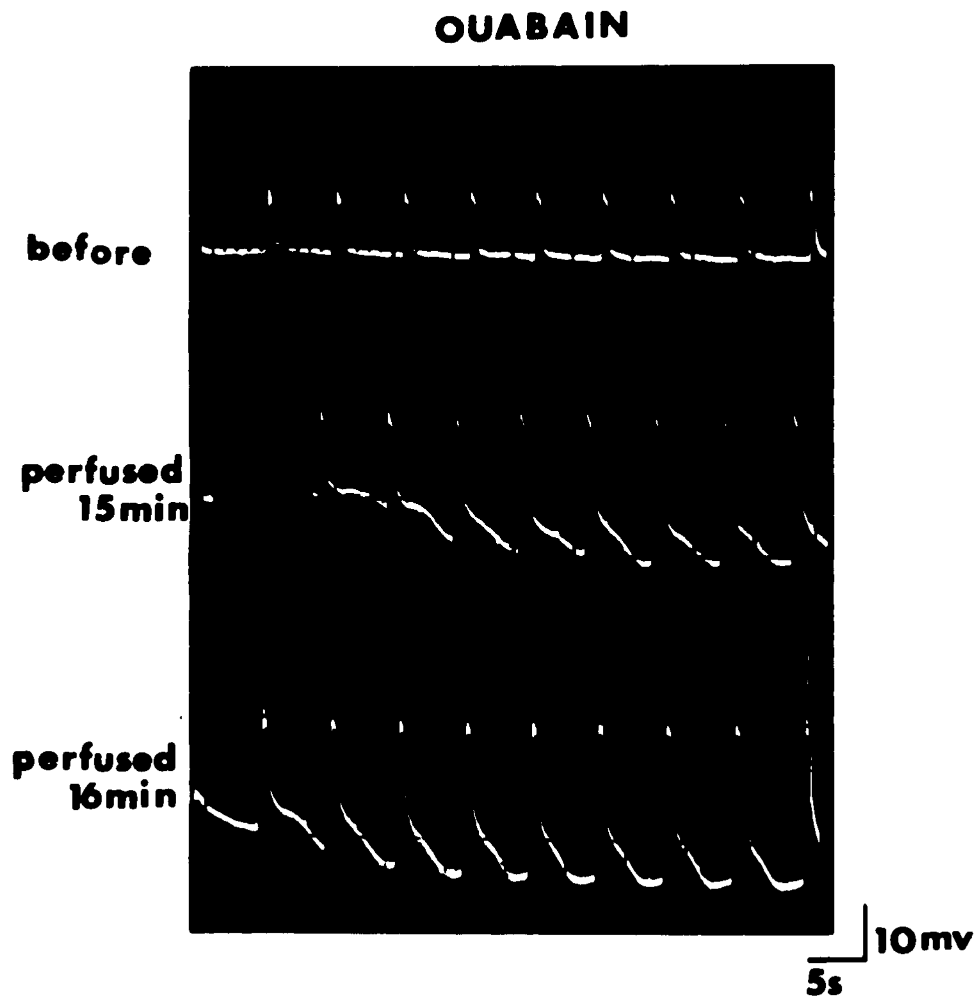


Figure 82

Figure 83

Model describing local circuit feedback loop in the ocellar retina of the dragonfly. Receptor terminals and L-neuron dendrites are indicated by the letters R and L respectively. Open arrows represent facilitatory transmitter action; black arrows represent inhibitory transmitter action. The direction of the arrows point from a pre-synaptic process to a post-synaptic process; the number of dots represent the relative amounts of neurotransmitter released during the various stages of the light response. The responses are separated into six different phases (A-F) which are represented by the darkened portion of the intracellular responses shown to the left of each section of the diagram. The phases of the light response correspond to:

(A) The dark adapted state.

(B) The period immediately following light-ON (ON-transient).

(C) The cutback from ON-transient to plateau.

(D) The sustained (plateau) portion of the response.

(E) The period immediately following light-OFF ("fast OFF-transient").

(F) The phase extending from the period immediately following light-OFF (E) to approximately 800 msec following light-OFF ("slow OFF-transient") which precedes the re-establishment of the dark equilibrium condition.

See text for explanation.

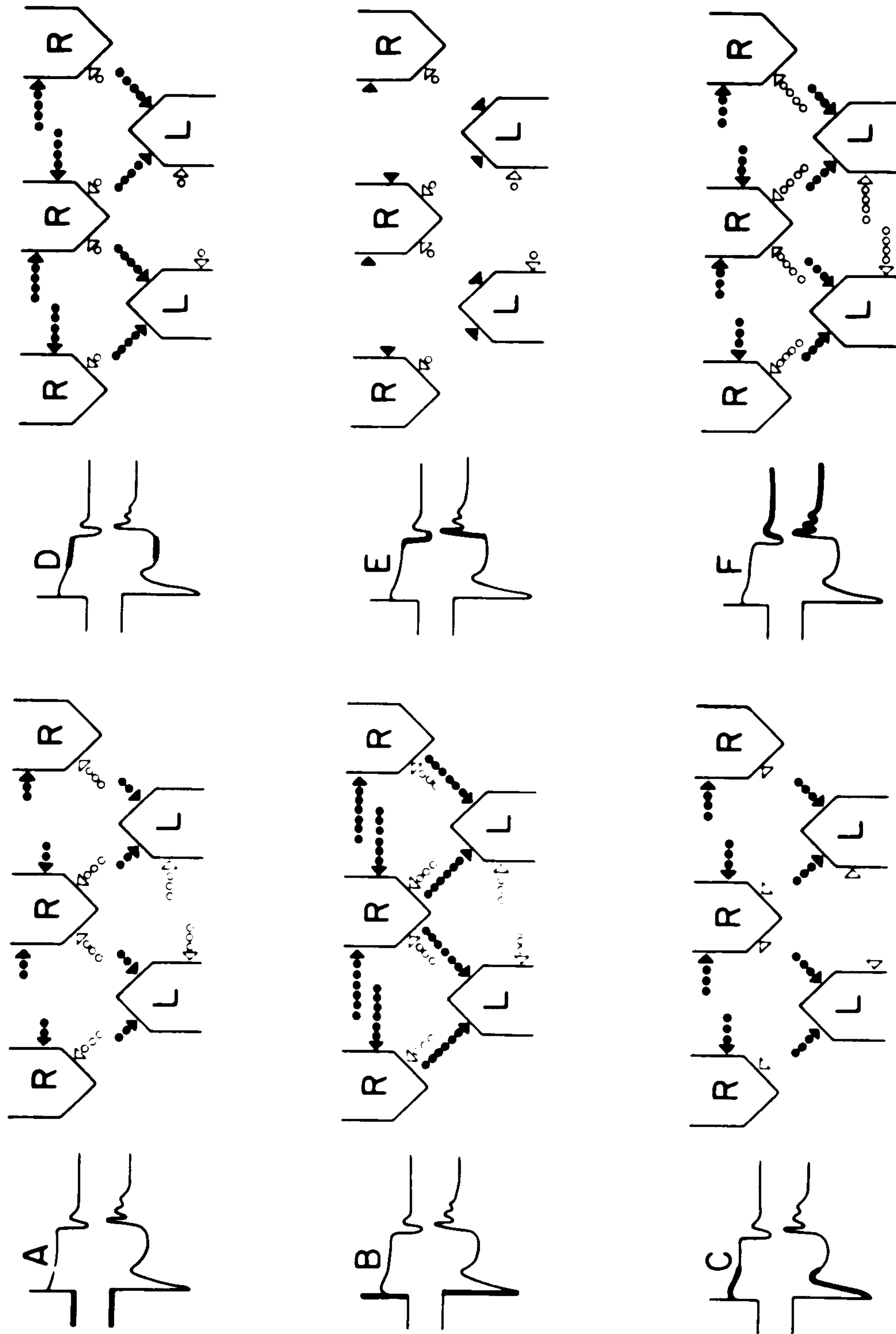


Figure 83

GRAPHS

The data shown on the following graphs was measured on an oscilloscope from tape recordings of selected experiments. In the case of those graphs depicting the responses of ocellar retinal neurons to constant intensity flashes, the values plotted represent the average of 3 to 5 responses. In the case of intensity-response series, 1 response at each stimulus intensity was measured.

The receptor OFF response was measured in 2 ways:

(1) The amplitude of the OFF hyperpolarization was measured with respect to the dark potential prior to light ON. Consequently, the amplitude of the OFF response (in millivolts) is shown as a negative value.

(2) The amplitude of the receptor OFF transient was measured from the sustained portion of the light response to the point of maximum negativity at light OFF. Therefore, the amplitude of the receptor OFF response is represented by its absolute value (in millivolts). The latter method was used more frequently because the interflash dark potential often fluctuated during drug perfusion experiments, but the level of the sustained depolarization during illumination did not vary (see pp. 114-115).

The L-neuron response was measured with respect to the pre-flash dark potential. The hyperpolarizing ON-transient and sustained components of the light response are given as negative values (in millivolts) while the depolarizing OFF-transient is represented by positive values (in millivolts).

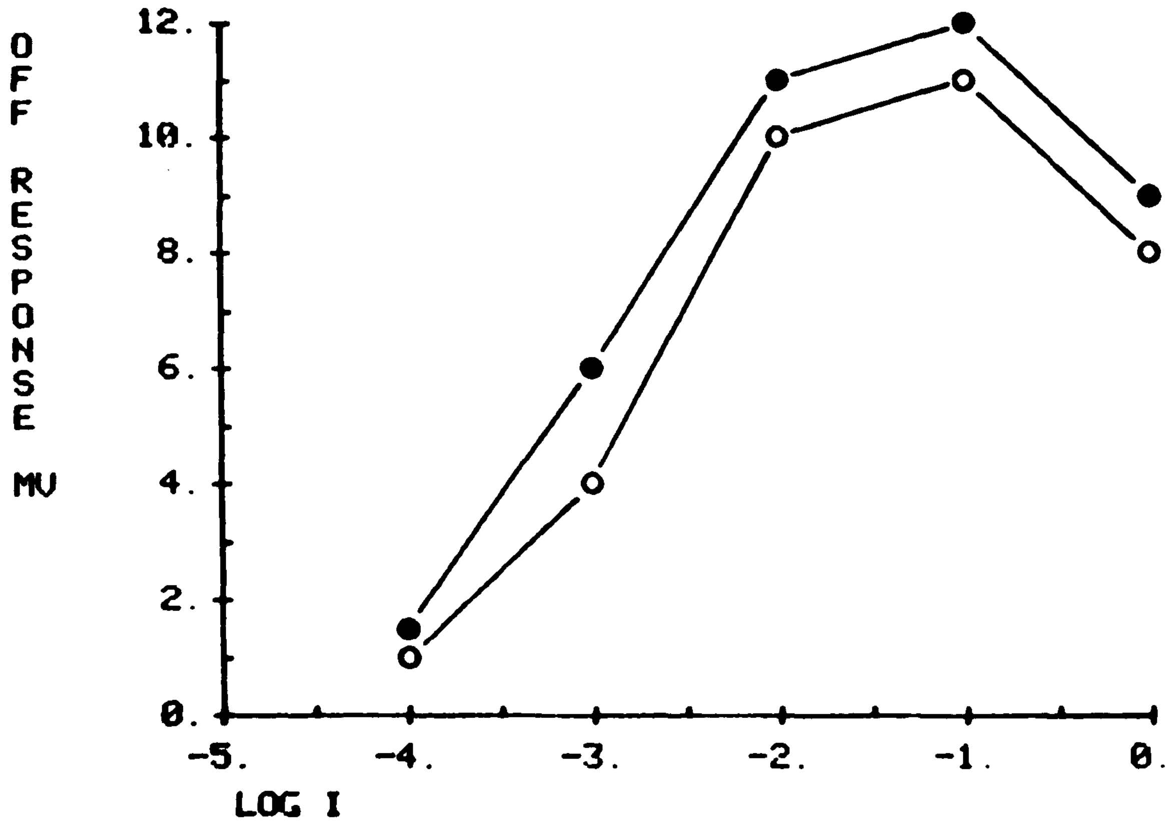
In several graphs, the change in the L-neuron dark potential is plotted. This value was obtained by measuring the change in the dc level of the baseline of the oscilloscope trace at various intervals during the time course of a drug perfusion experiment. The significance of this value is inconclusive because several factors (in addition to a change in transmembrane potential) might contribute to the observed change in the dc level of the recording (see pp. 114-115). In all graphs, the dark potential before drug perfusion corresponds to 0 millivolts on the ordinate scale. This value represents the dark adapted "resting" potential before drug perfusion was initiated. However, when the change in dark potential is shown on the same graph as the L-neuron response to light, the dark potential corresponds to the change in the dc level of the recording while the L-neuron response corresponds to the amplitude of response with respect to the dark potential before light-ON. The latter curves were not adjusted for changes in the baseline of the recording.

Graph I

Amplitude of receptor OFF response plotted as a function of stimulus intensity. This data was obtained from the experiments shown in figures 2 and 3. The OFF response was largest following moderate intensity stimuli.

Ordinate: Amplitude of receptor OFF response (mv) measured from the sustained portion of the light response ("mv from sust"). This component of the receptor OFF response corresponds to phase E in figure 83.

Abscissa: Intensity of illumination (Log I).



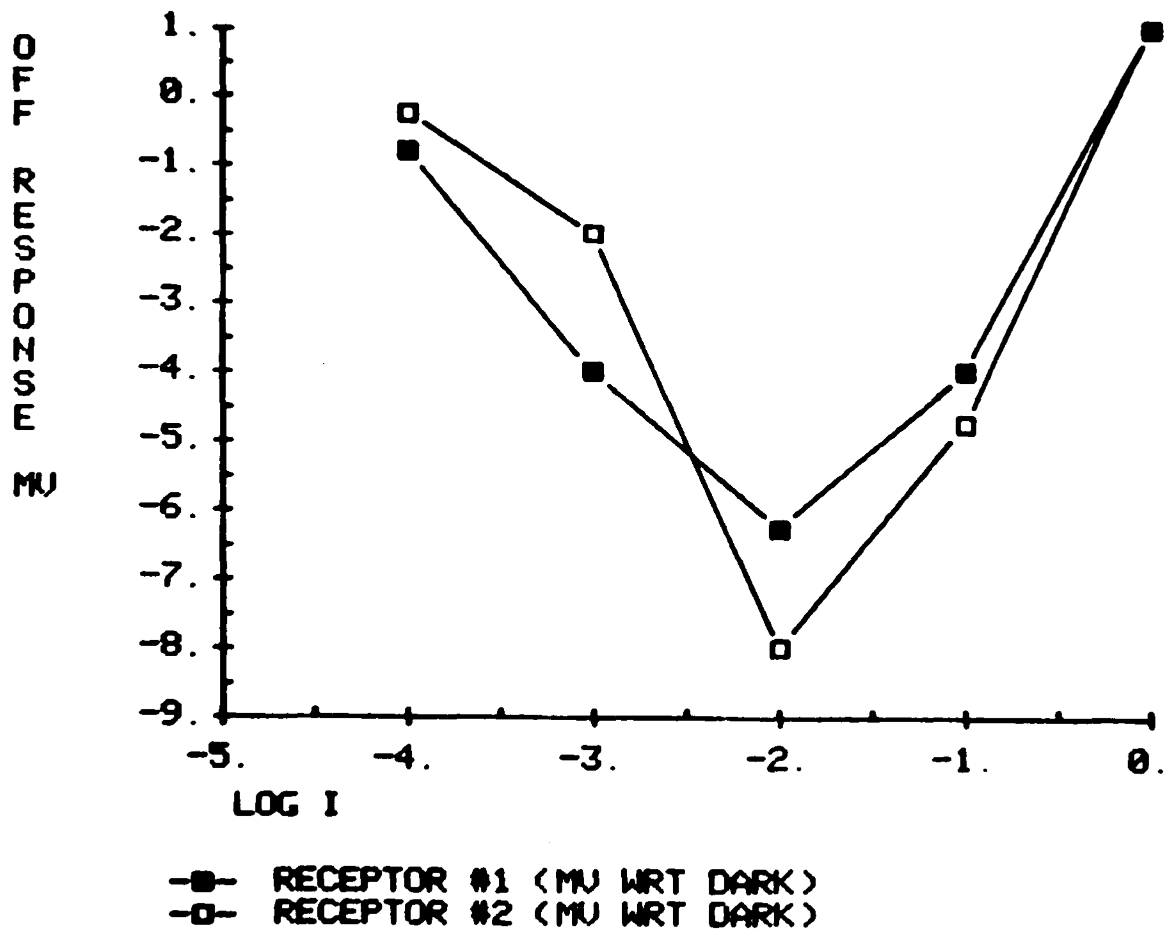
Graph I

Graph II

Amplitude of receptor OFF response plotted as a function of stimulus intensity. This data was obtained from the experiments shown in figures 2 and 3. The OFF hyperpolarization was largest following moderate intensity light stimuli.

Ordinate: Amplitude of receptor OFF hyperpolarization (mv) measured with respect to the dark potential ("mv wrt dark"). The dark adapted ("resting") potential corresponds to 0 millivolts on the ordinate scale.

Abscissa: Intensity of illumination (Log I).



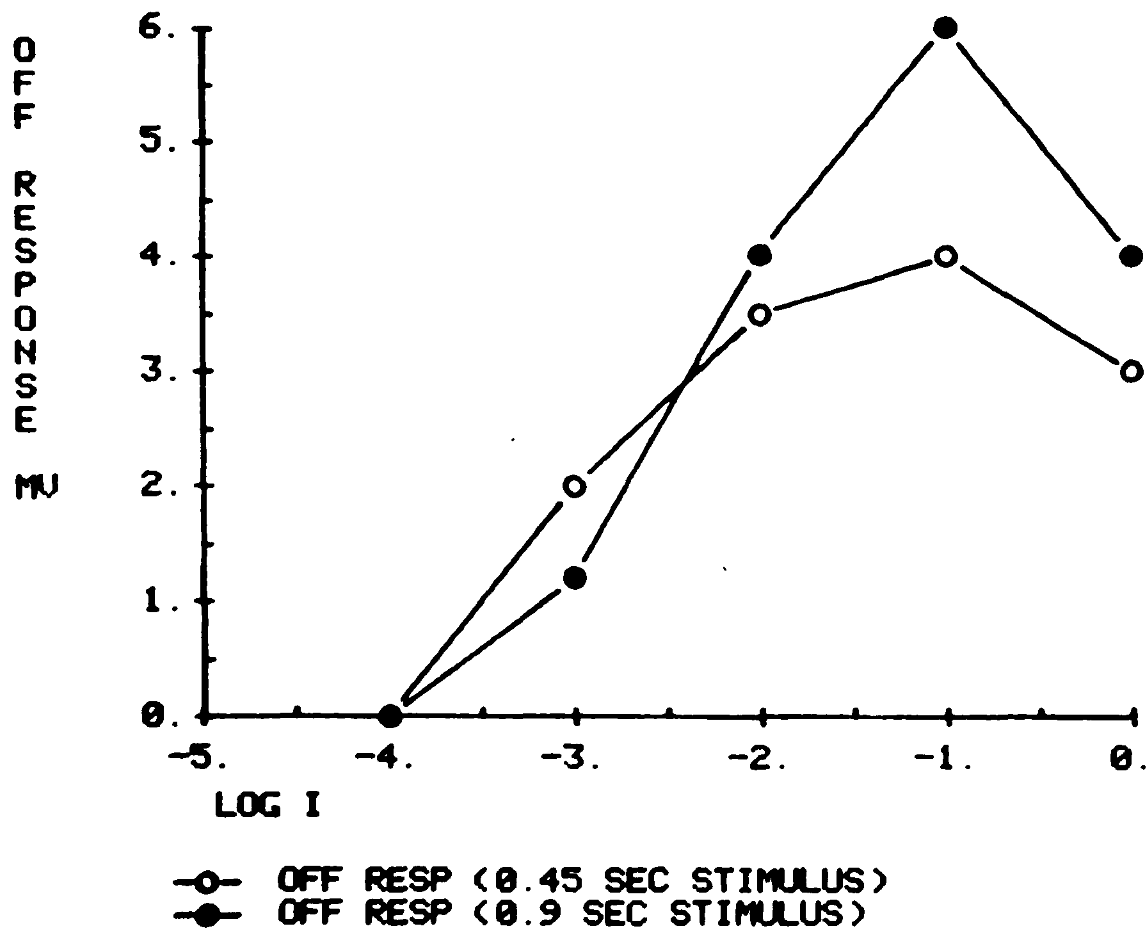
Graph II

Graph III

Effect of increased duration light stimulus on OFF response recorded from receptor terminal. The amplitude of the receptor OFF response is plotted as a function of stimulus intensity. This data was obtained from the experiment shown in figures 5, 8, 9, and 10 (see figure 10). The two curves represent the OFF transient in response to brief (0.45 sec) and prolonged (0.9 sec) light stimuli. The intensity-response series to the long duration test flash was recorded from a relatively light-adapted preparation (after 2 minutes of continuous Log I = -1 test flashes; see figure 9). The intensity-response series to the brief test flash was recorded after 2 minutes of dark adaptation (see figures 5 and 9). Increasing the duration of the light stimulus increased the amplitude of the OFF response for stimuli brighter than Log I = -2.

Ordinate: Amplitude of OFF response (mv) measured from the sustained portion of the light response. This component of the response corresponds to phase E in figure 83.

Abscissa: Intensity of illumination (Log I).



Graph III

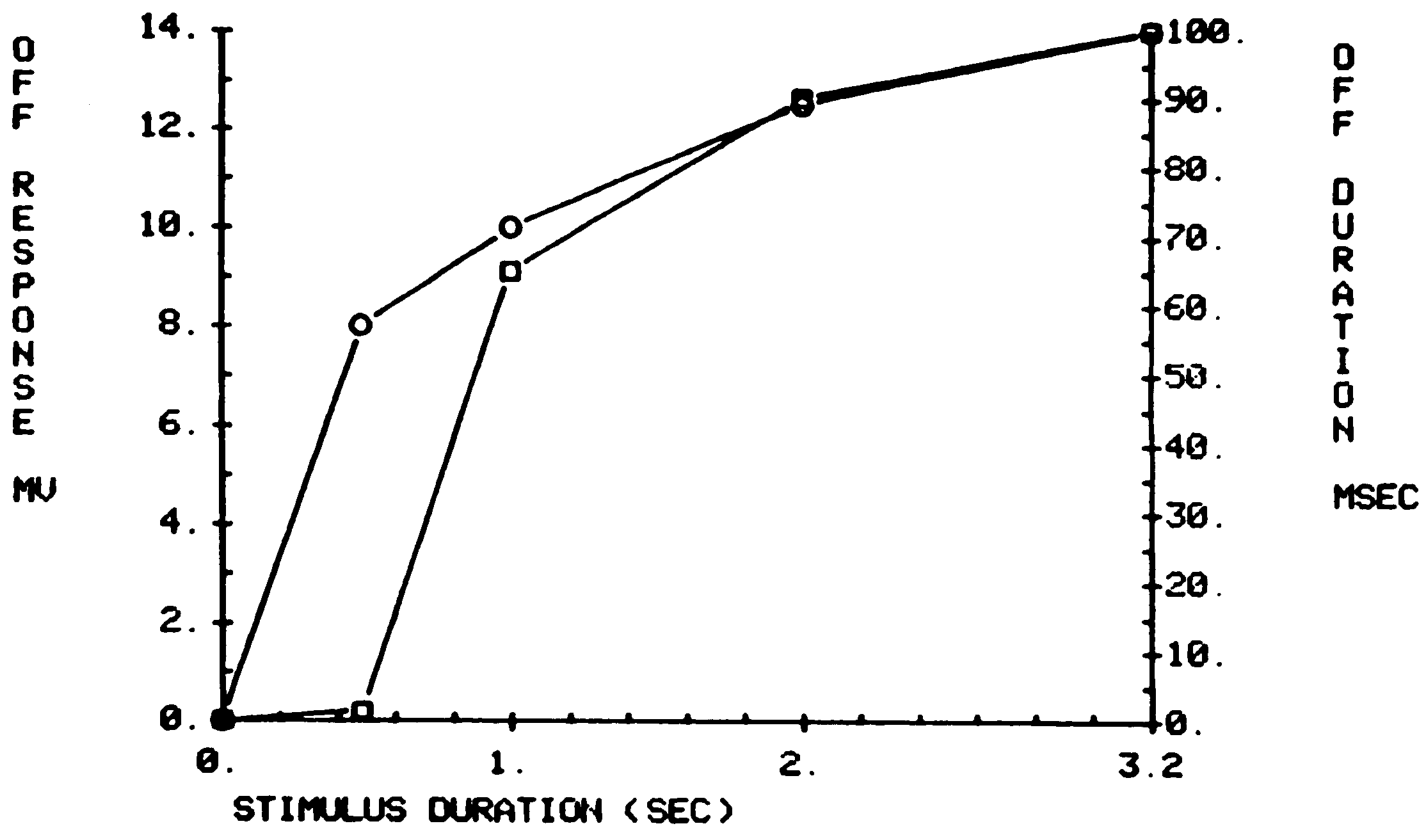
Graph IV

Amplitude and duration of receptor OFF response plotted as a function of stimulus intensity. This data was obtained from the experiment shown in figure 14. The amplitude and duration of the receptor OFF response increased as the duration of the light stimulus was increased.

Stimulus intensity: Log I = 0.

Ordinate: Amplitude of receptor OFF transient (mv) measured from the sustained component of the light response ("mv from sust"). This component of the receptor response corresponds to phase E in figure 83.

Abscissa: Duration of light stimulus (seconds).



Graph IV

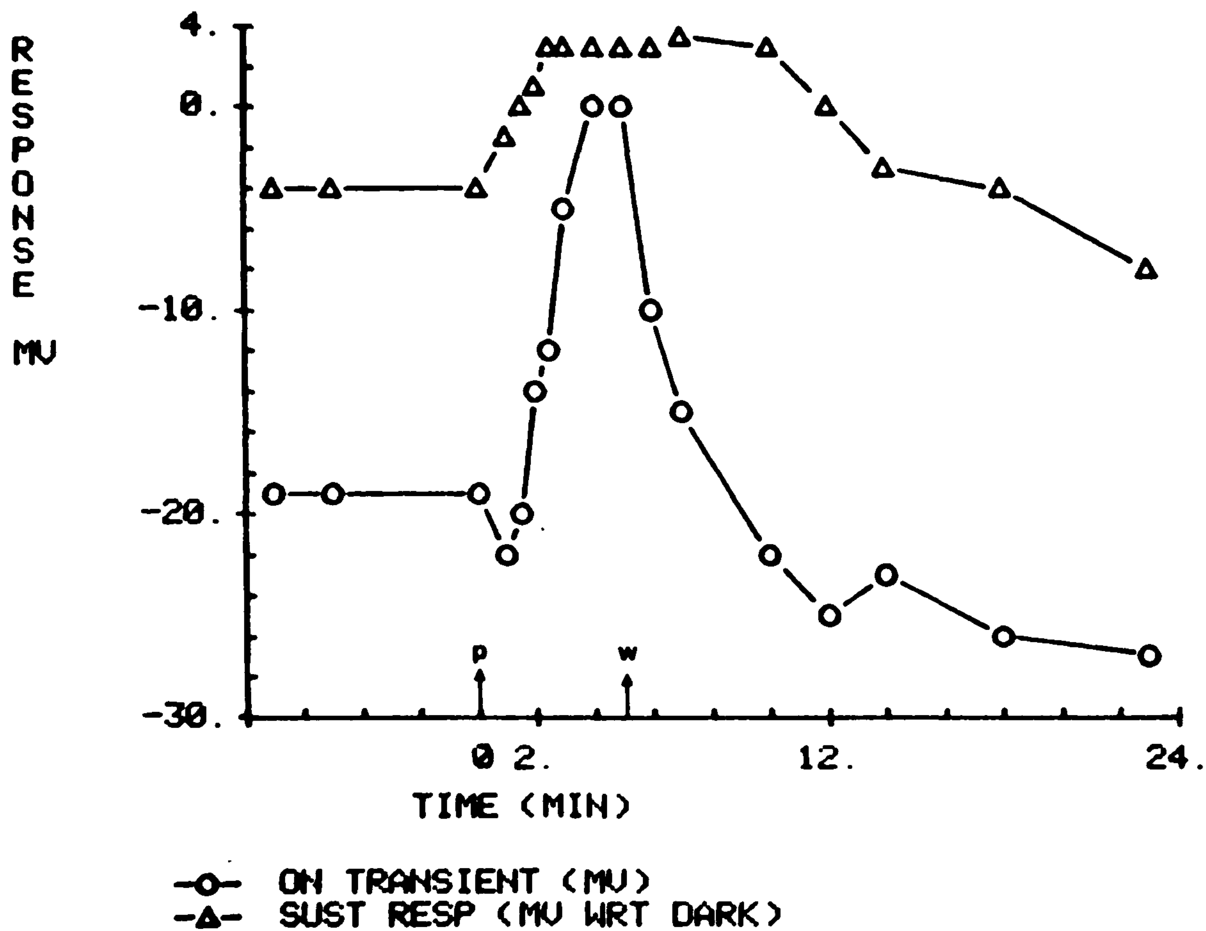
Graph V

Curare perfusion experiment. Effect of 1.4 mM curare on ON-transient (see B in figure 83) and sustained (see D in figure 83) components of L-neuron response. This data was obtained from the experiment shown in figure 42. The amplitude of the response (millivolts with respect to pre-flash dark potential) is plotted as a function of time. Curare reversibly blocked both hyperpolarizing components of the L-neuron response. Note that during the application of curare, the sustained response was eliminated and became positive with respect to the pre-flash dark potential (see figure 50 and pp. 50, 95-96).

Stimulus intensity: Log I = -2.

Ordinate: Amplitude of ON-transient and sustained components of L-neuron light response, measured with respect to the pre-flash dark potential ("mv wrt dark"). The dark potential at the time perfusion was initiated corresponds to 0 millivolts on the ordinate scale.

Abscissa: Time course of curare perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



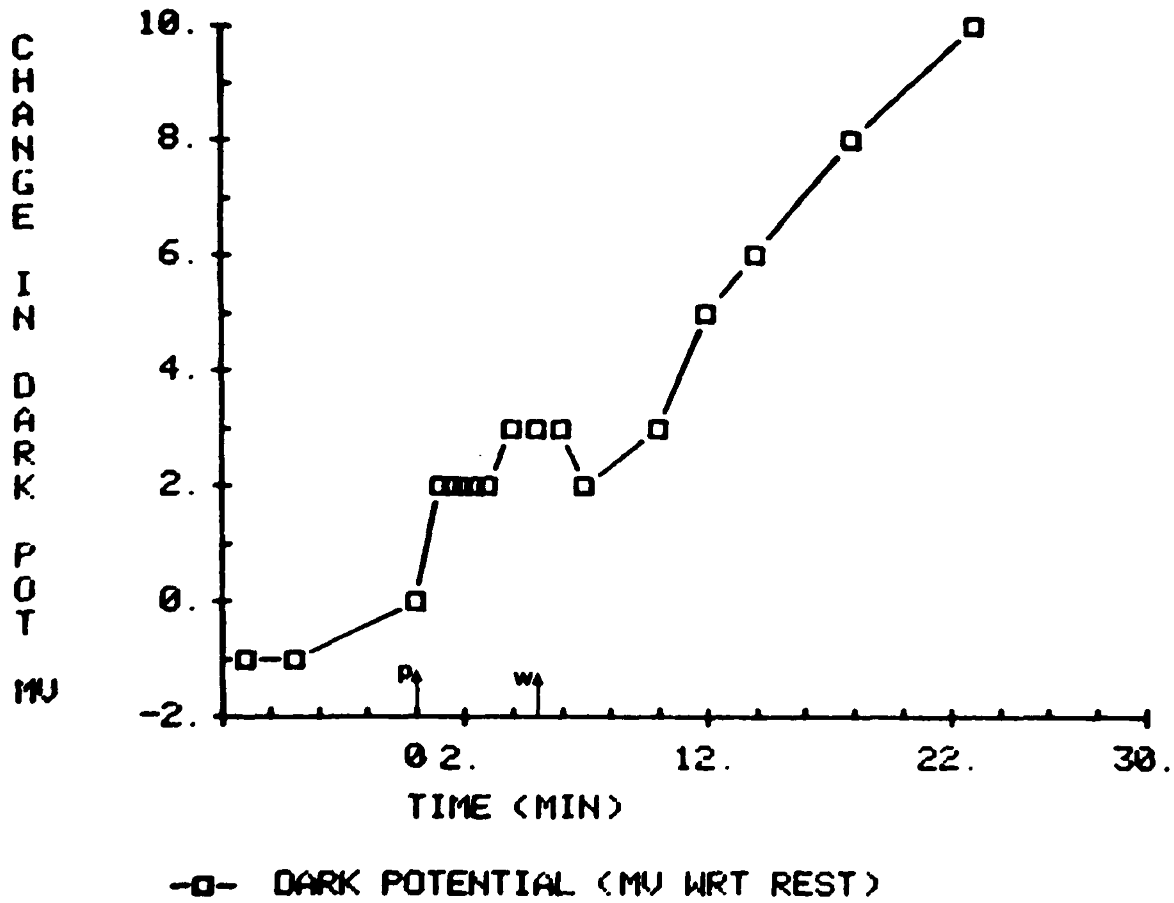
Graph V

Graph VI

Effect of 1.4 mM curare on dark potential (see A in figure 83) of L-neuron. The change in dark potential (with respect to the dark "resting" potential prior to curare perfusion) is plotted as a function of time. This data was obtained from the experiment shown in figures 42 and 43. During the application of curare the dark potential depolarized but did not recover during the wash period. The electrode was withdrawn before the potential stabilized. (see also Graph XI where the dark potential stabilized after depolarizing 16 mv).

Ordinate: Change in L-neuron dark potential ("mv wrt rest"). The dark "resting" potential prior to the application of curare corresponds to 0 millivolts on the ordinate scale.

Abscissa: Time course of curare perfusion experiment (minutes). Perfusion and wash times are dindicated by arrows.



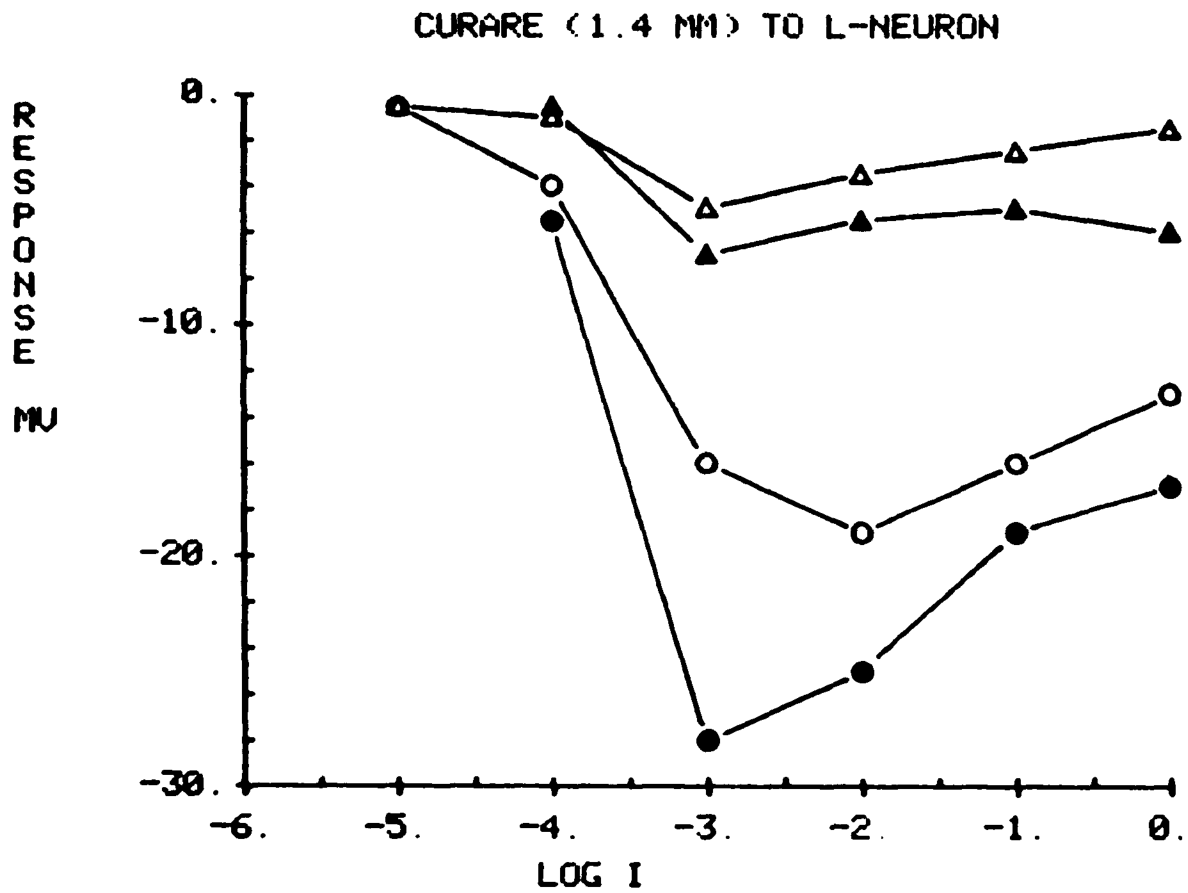
Graph VI

Graph VII

Effect of 1.4 mM curare on ON-transient (see B in figure 83) and sustained (see D in figure 83) components of L-neuron response. This data was obtained from the experiment shown in figures 42 and 43. The amplitude of the response (measured with respect to the pre-flash dark potential) is plotted as a function of stimulus intensity (Log I). Intensity-response series were measured before curare perfusion and 13 minutes after Ringer wash was initiated. The amplitude of the ON-transient and sustained components of the L-neuron response increased during the wash period.

Ordinate: Amplitude of response with respect to the pre-flash dark potential, which corresponds to 0 mv on the ordinate scale.

Abscissa: Intensity of illumination (Log I).



Graph VII

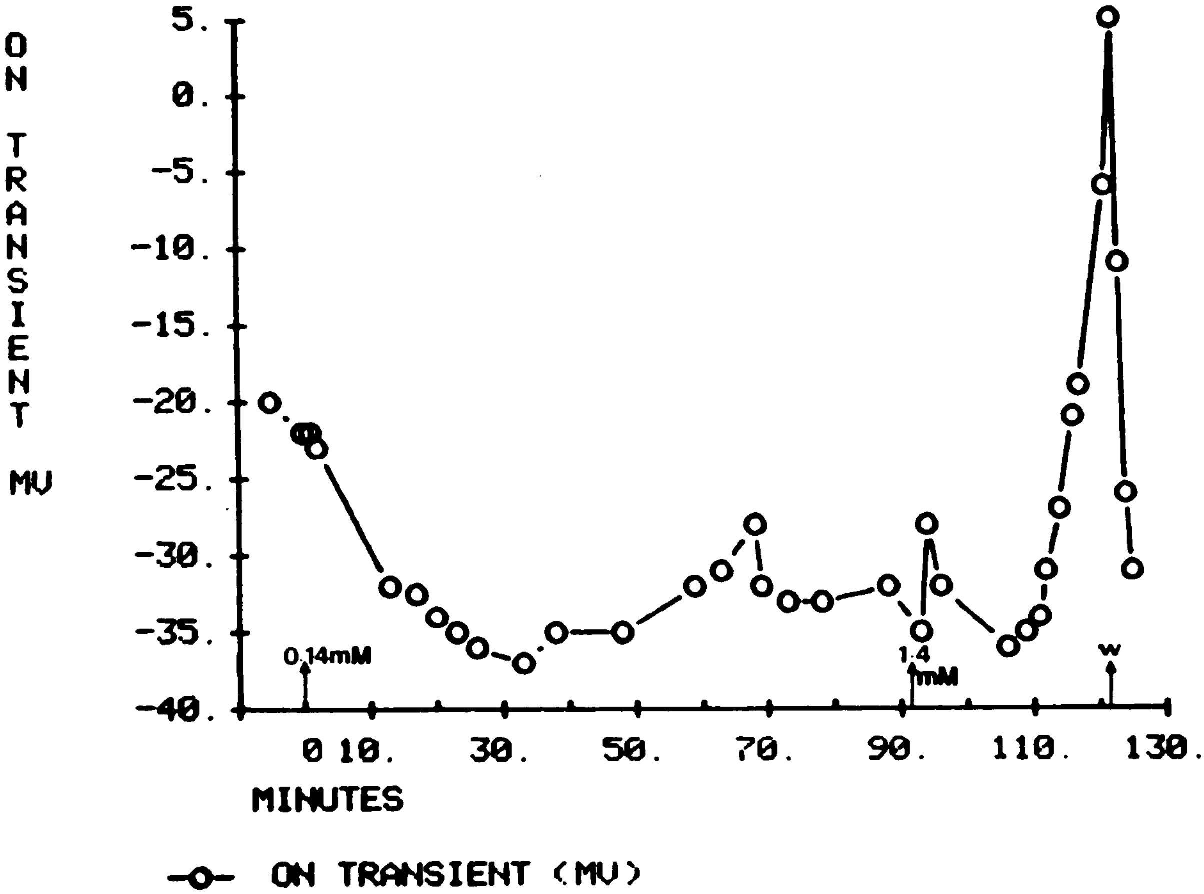
Graph VIII

Curare perfusion experiment. Time course of change in ON-transient component (see B in figure 83) of L-neuron response during 2 consecutive applications of curare (0.14 mM and 1.4 mM). The change in the amplitude of the hyperpolarizing ON-transient L-neuron response is plotted as a function of time. This data was obtained from the experiment shown in figure 46. The ON-transient increased in amplitude during the application of 0.14 mM curare but was eliminated during perfusion with 1.4 mM curare.

Stimulus intensity: Log I = -2.

Ordinate: Amplitude of ON-transient L-neuron response with respect to the dark potential prior to light-ON (see A in figure 83). The dark potential corresponds to 0 mv on the ordinate scale.

Abscissa: Time course of drug perfusion experiment. Perfusion and wash times are indicated by arrows.



Graph VIII

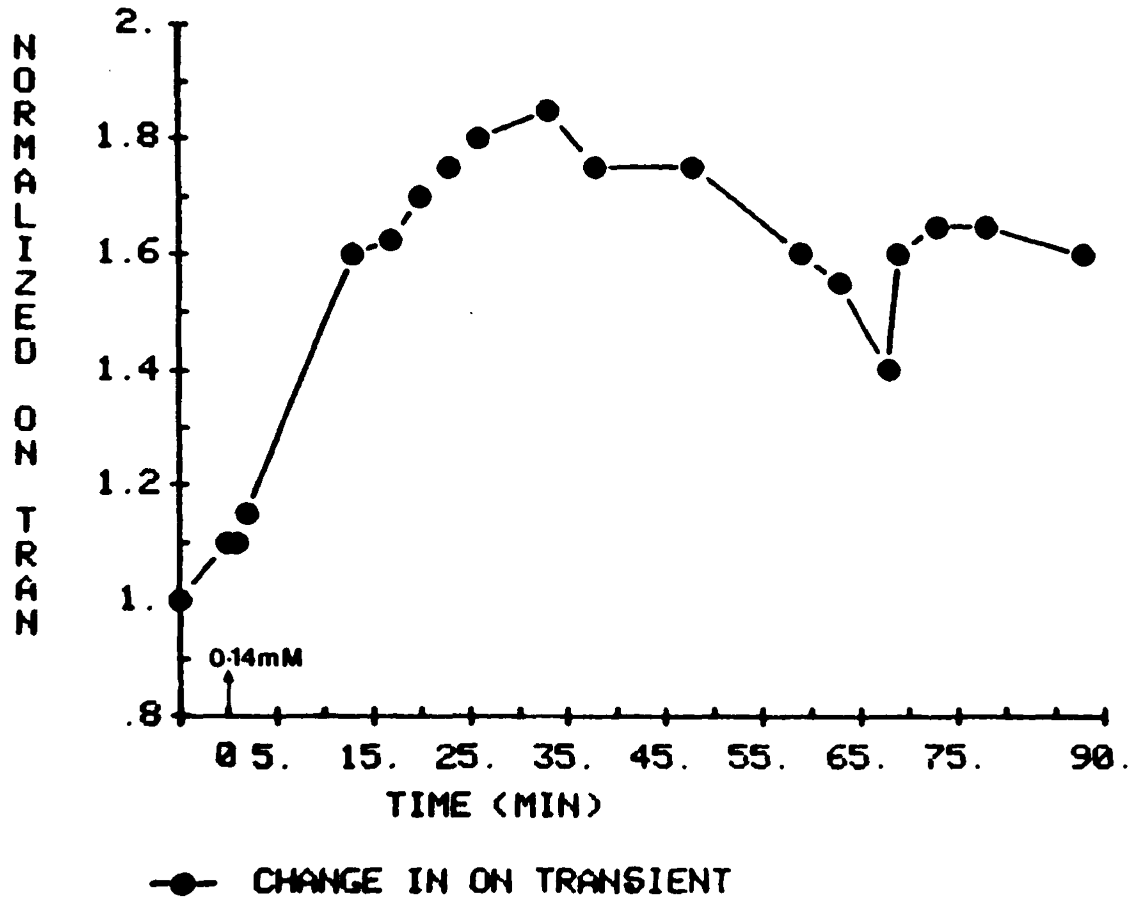
Graph IX

Curare perfusion experiment. Time course of relative change in ON-transient component (see B in figure 83) of L-neuron response during the application of 0.14 mM curare. The hyperpolarizing ON-transient response to light was normalized by dividing all responses (in mv) by the magnitude of the response (-20 mv) just before curare perfusion was initiated. The normalized L-neuron ON-transient response is plotted as a function of time. This data was obtained from the experiment shown in figures 46 and 47. The ON-transient almost doubled in amplitude during the early stages of curare perfusion. Subsequent application of 1.4 mM curare eliminated the ON-transient response to light (see Graph VIII).

Stimulus intensity: Log I = -2.

Ordinate: Normalized ON-transient response of L-neuron.

Abscissa: Time course (in minutes) of curare perfusion experiment. Curare perfusion was initiated at time 0 on the abscissa scale.



Graph IX

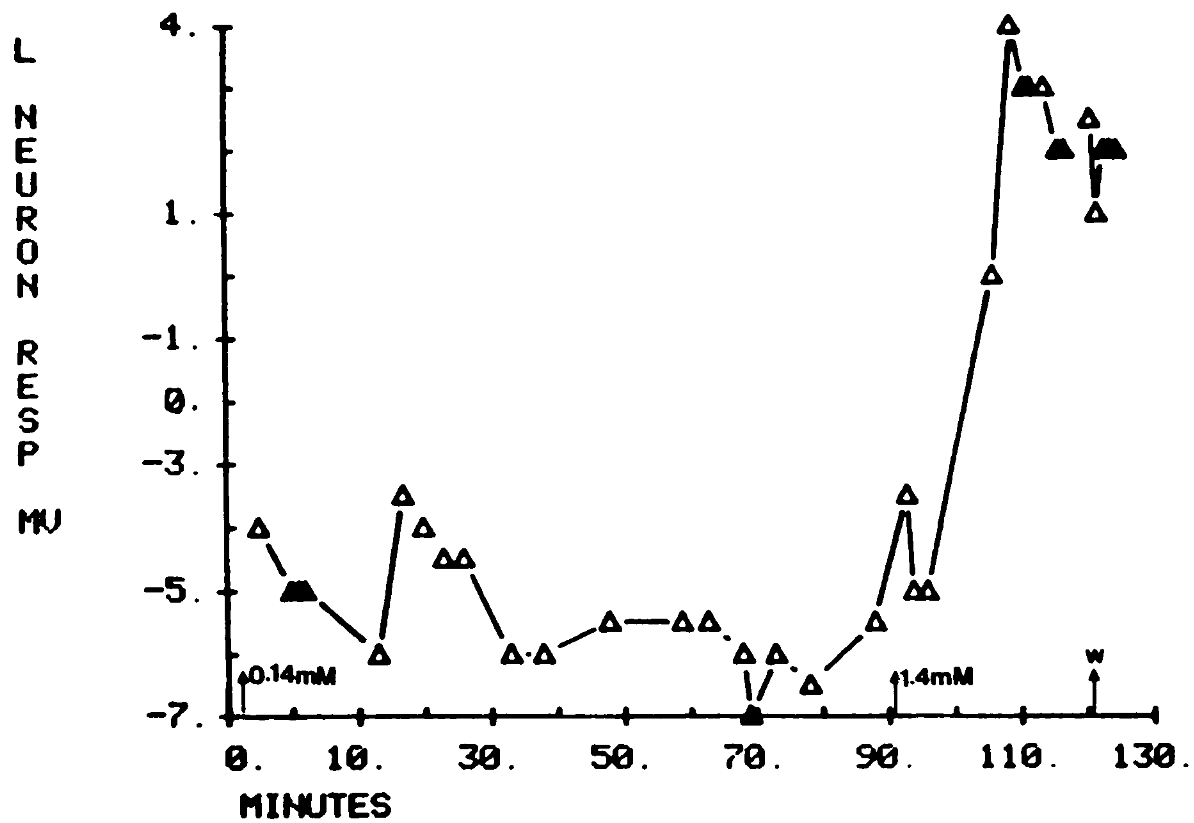
Graph X

Curare perfusion experiment. Time course of relative change in sustained component (see D in figure 83) of L-neuron response during two consecutive applications of curare (0.14 mM followed by 1.4 mM). The change in the sustained L-neuron response is plotted as a function of time. The sustained response to light was not eliminated during perfusion with the lower dose (0.14 mM) but was blocked during the application of the more concentrated solution (1.4 mM). This data was obtained from the experiment shown in figures 46 and 47 (see also Graphs VIII-IX).

Stimulus intensity: Log I = -2.

Ordinate: Amplitude of sustained L-neuron response (mv) with respect to the dark potential prior to light-ON (see A in figure 83). The dark potential corresponds to 0 mv on the ordinate scale.

Abscissa: Time course (in minutes) of curare perfusion experiment. Perfusion and wash times are indicated by arrows.



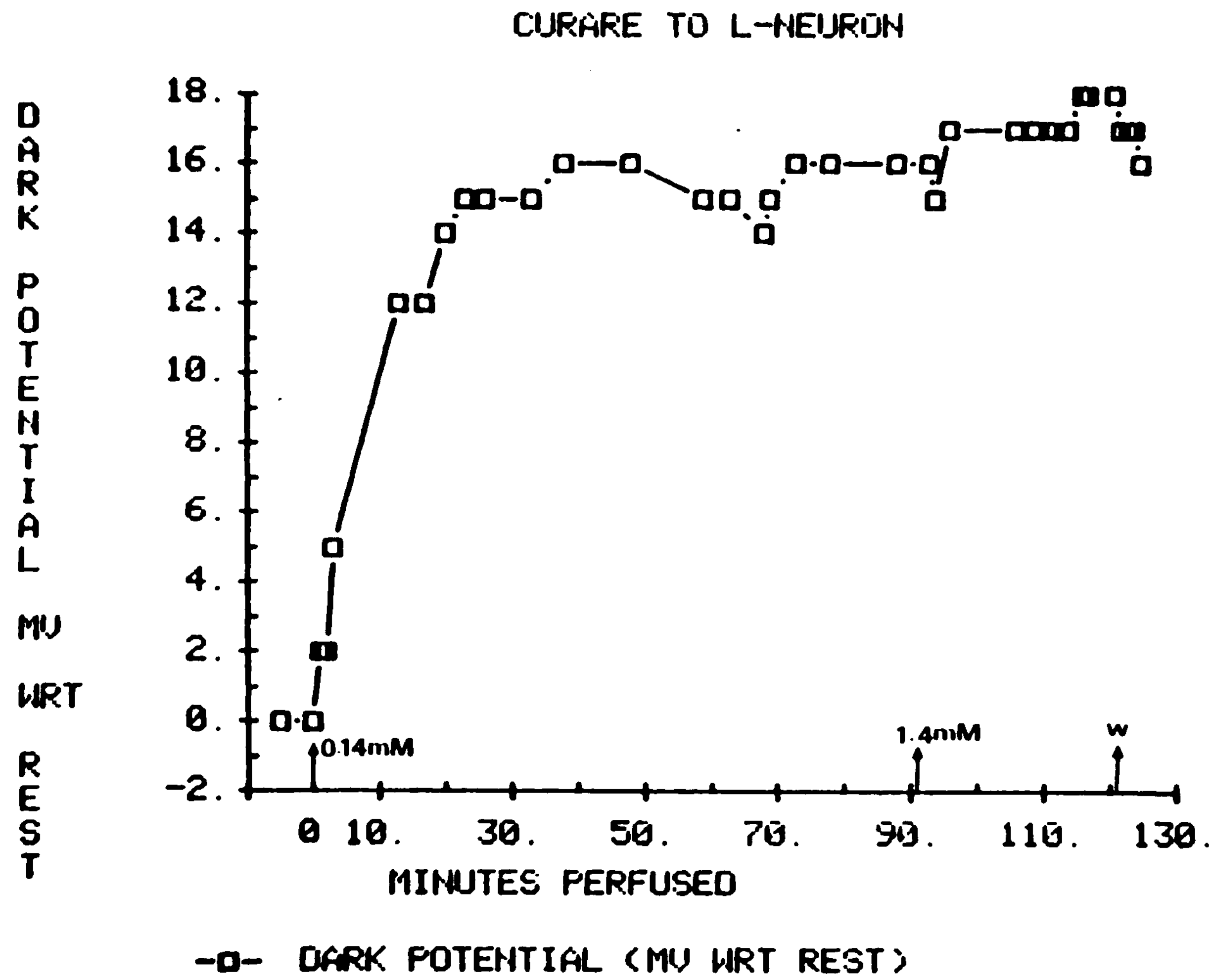
Graph X

Graph XI

Curare perfusion experiment. Time course of change in L-neuron dark potential (see A in figure 83) during two consecutive applications of curare (0.14 mM and 1.4 mM). The change in dark potential (mv) is plotted as a function of time. This data was obtained from the experiments shown in figures 46 and 47. The dark potential depolarized during curare perfusion.

Ordinate: Change in dark potential during curare perfusion ("mv wrt rest"). The dark ("resting") potential at the time drug perfusion was initiated corresponds to 0 mv on the ordinate scale.

Abscissa: Time course of curare perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XI

Graph XII

Curare perfusion experiment. Effect of 2 consecutive applications of curare (0.14 mM and 1.4 mM) on the dark potential (see A in figure 83), ON-transient (see B in figure 83) and sustained (see D in figure 83) phase of the L-neuron response. The changes in the L-neuron potential are plotted as a function of time (see Graphs VIII-XI). This data was obtained from the experiment shown in figures 46 and 47. The 3 curves represent the change in dark potential (with respect to the dark "resting" potential before curare perfusion), the sustained response (millivolts with respect to the pre-flash dark potential)*, and the ON-transient response (millivolts with respect to the pre-flash dark potential).*

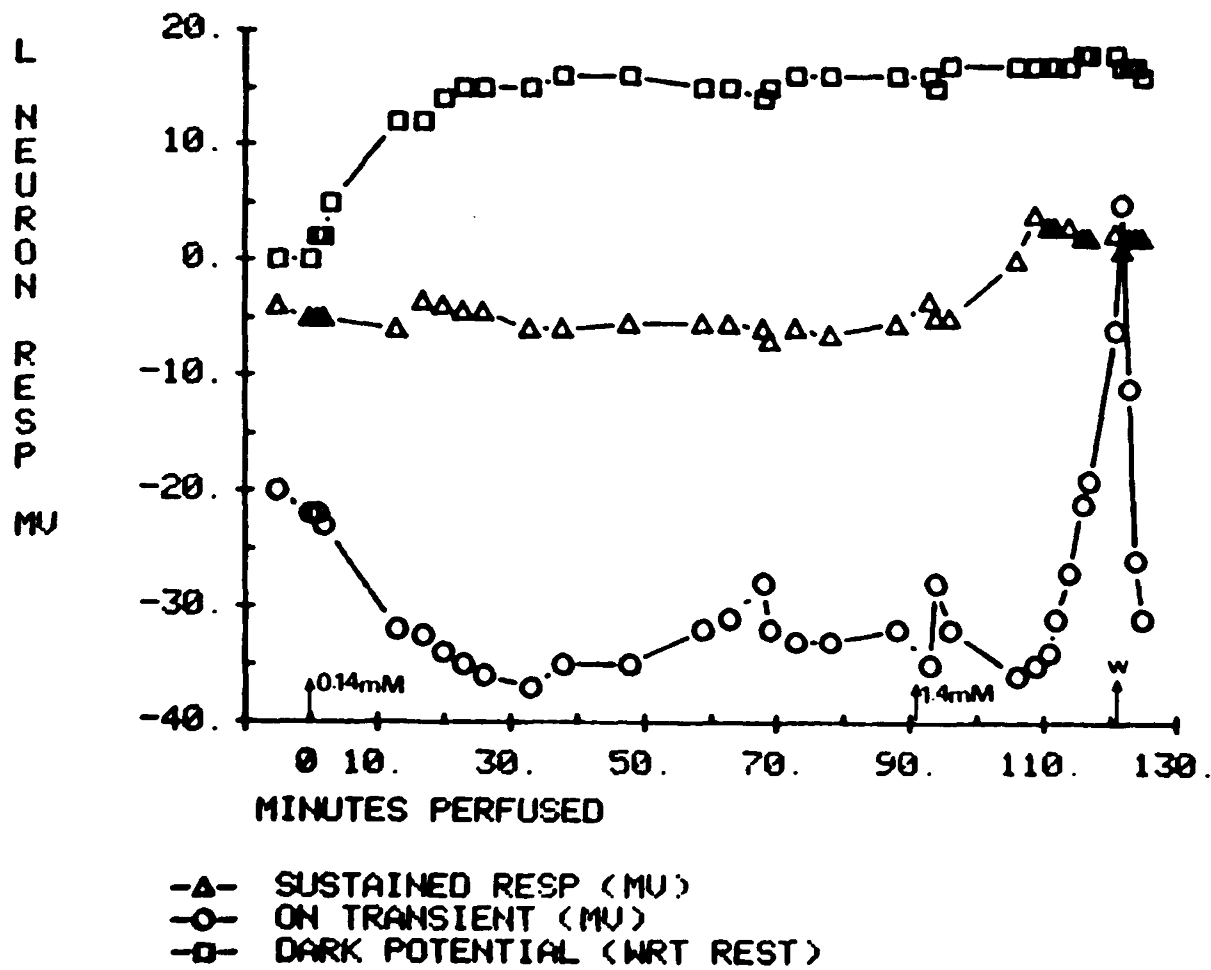
<u>Stimulus intensity:</u> Log I = -2.

<u>Ordinate:</u> Amplitude of L-neuron response (millivolts). The dark potential prior to curare perfusion corresponds to 0 millivolts on the ordinate scale.

<u>Abscissa:</u> Time course of curare perfusion experiment (minutes). See figures 46 and 47 and Graphs VIII, X, and XI. Perfusion and wash times are indicated by arrows.

* The magnitude of the light response components with respect to the dark potential before curare perfusion can be estimated therefore, by adding the upper (inter-flash dark potential) curve. For example, the potential reached by the ON-transient was almost unchanged during 0.14 mM curare perfusion although its amplitude increased with respect to the interflash dark potential, as shown here.

CURARE TO L-NEURON



Graph XII

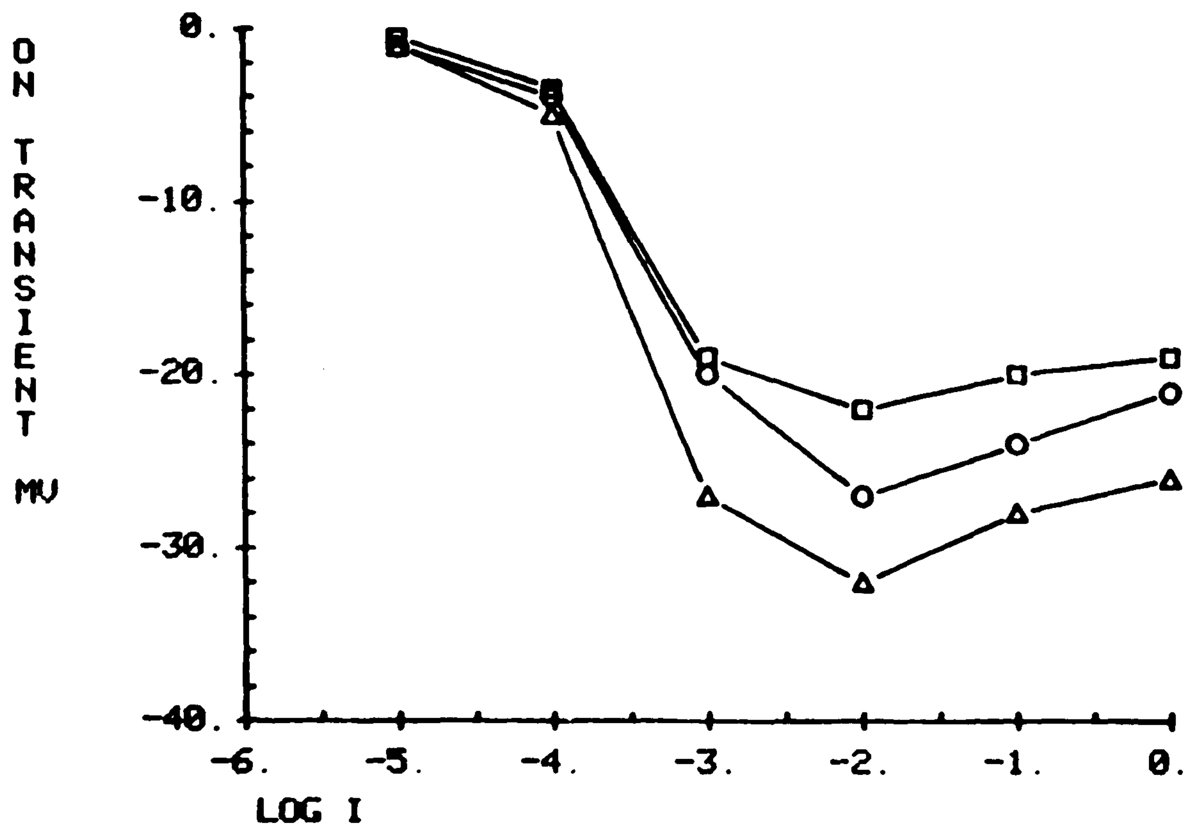
Graph XIII

Effect of 0.14 mM curare on ON-transient component (see B in figure 83) of L-neuron light response. The ON-transient is plotted as a function of stimulus intensity (Log I). This data was obtained from the experiment shown in figure 48. The 3 curves represent intensity-response series recorded before and during curare perfusion (perfused for 3 min and 14 min respectively). Lower concentrations of curare increased the amplitude of the ON-transient response of the L-neuron (see also Graphs VIII, IX and XII).

<u>Ordinate:</u> Amplitude of ON-transient response (mv) with respect to the dark potential. The dark potential corresponds to 0 mv on the ordinate scale.

<u>Abscissa:</u> Intensity of illumination (Log I).

CURARE TO L-NEURON



-□- BEFORE 0.14 MM CURARE
-○- 0.14 MM CURARE (3 MIN)
-△- 0.14 MM CURARE (14 MIN)

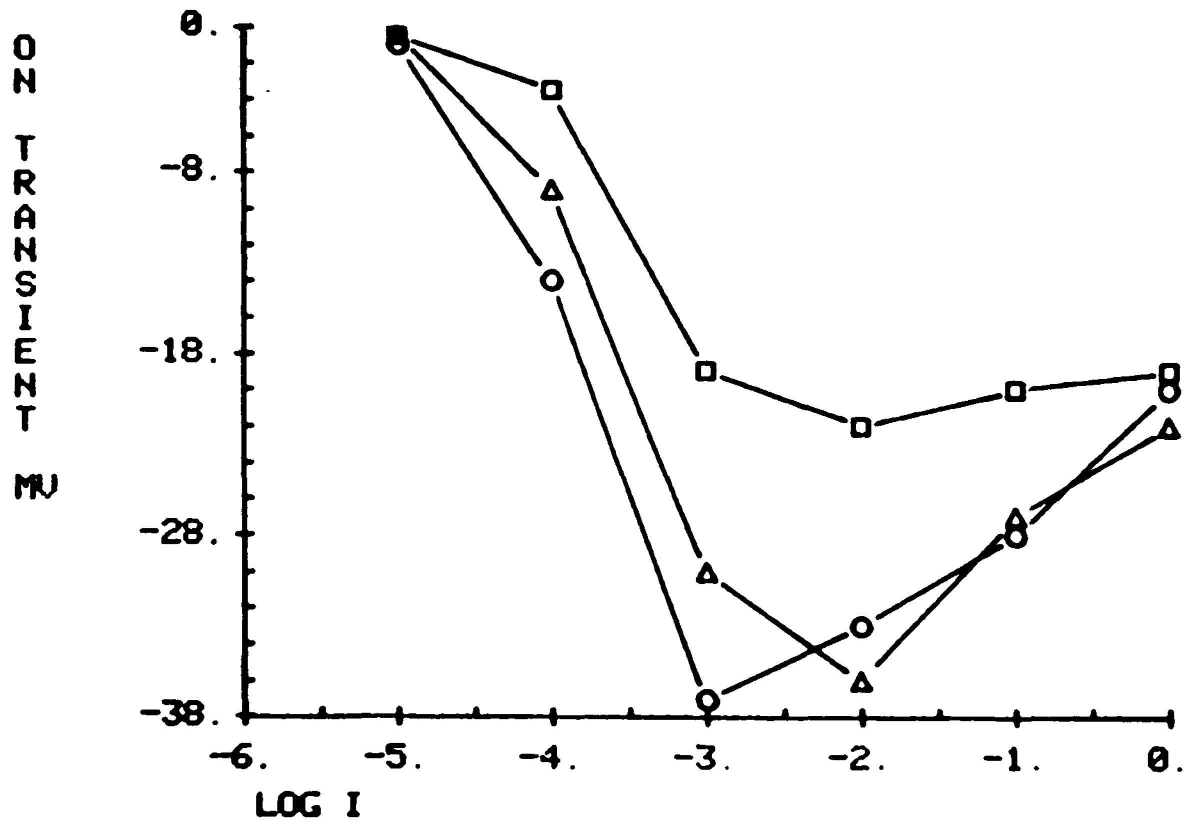
Graph XIII

Graph XIV

Effect of 2 concentrations of curare (0.14 mM and 1.4 mM) on the ON-transient component of the L-neuron light response. The ON-transient is plotted as a function of stimulus intensity (Log I). This data was obtained from the experiment shown in figure 48. The 3 curves represent intensity-response series recorded before and during 2 consecutive applications of curare (0.14 mM perfused for 88 min). The application of the higher concentration of curare (1.4 mM) started to reduce the amplitude of the ON-transient response of the L-neuron (see Graphs VIII, XII, and XIII).

Ordinate: Amplitude of ON-transient response with respect to the dark potential. The dark potential corresponds to 0 mv on the ordinate scale.

Abscissa: Intensity of illumination (Log I).



Graph XIV

Graph XV

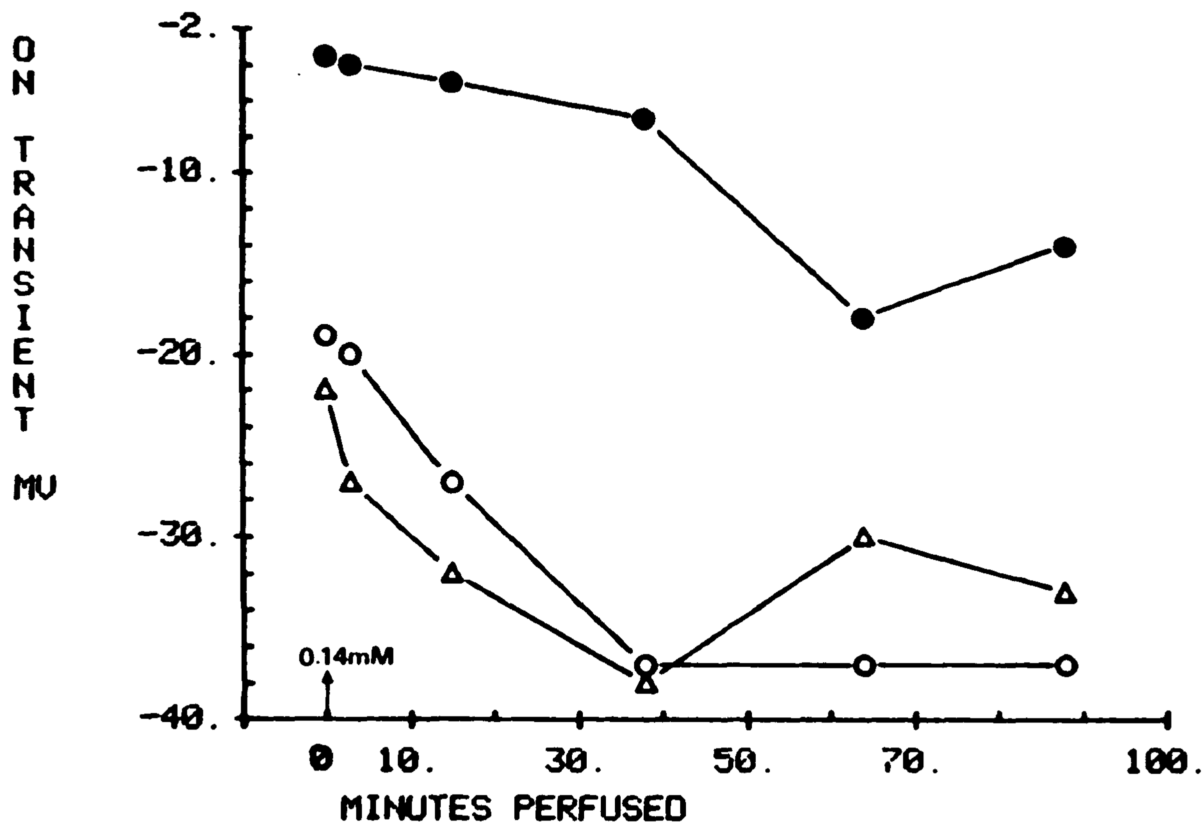
Effect of 0.14 mM curare on the hyperpolarizing ON-transient component (see B in figure 83) of the L-neuron light response. The ON-transient is plotted as a function of perfusion time. This data was obtained from the experiment shown in figure 48. The 3 curves represent the change in the ON-transient in response to 3 different stimulus intensities (Log I = -4, Log I = -3, Log I = -2).

During the early stages of curare perfusion the ON-transient increased in amplitude. This enhancement of the light response was especially prominent following less intense test flashes (see Graph XIII).

Ordinate: Amplitude of ON-transient response with respect to the dark potential. The dark potential corresponds to 0 mv and is not shown on the ordinate scale in this graph.

Abscissa: Perfusion time (minutes). Curare perfusion was initiated at time 0 on the abscissa.

CURARE TO L-NEURON



—●— LOG I=-4
—○— LOG I= -3
—△— LOG I= -2

Graph XV

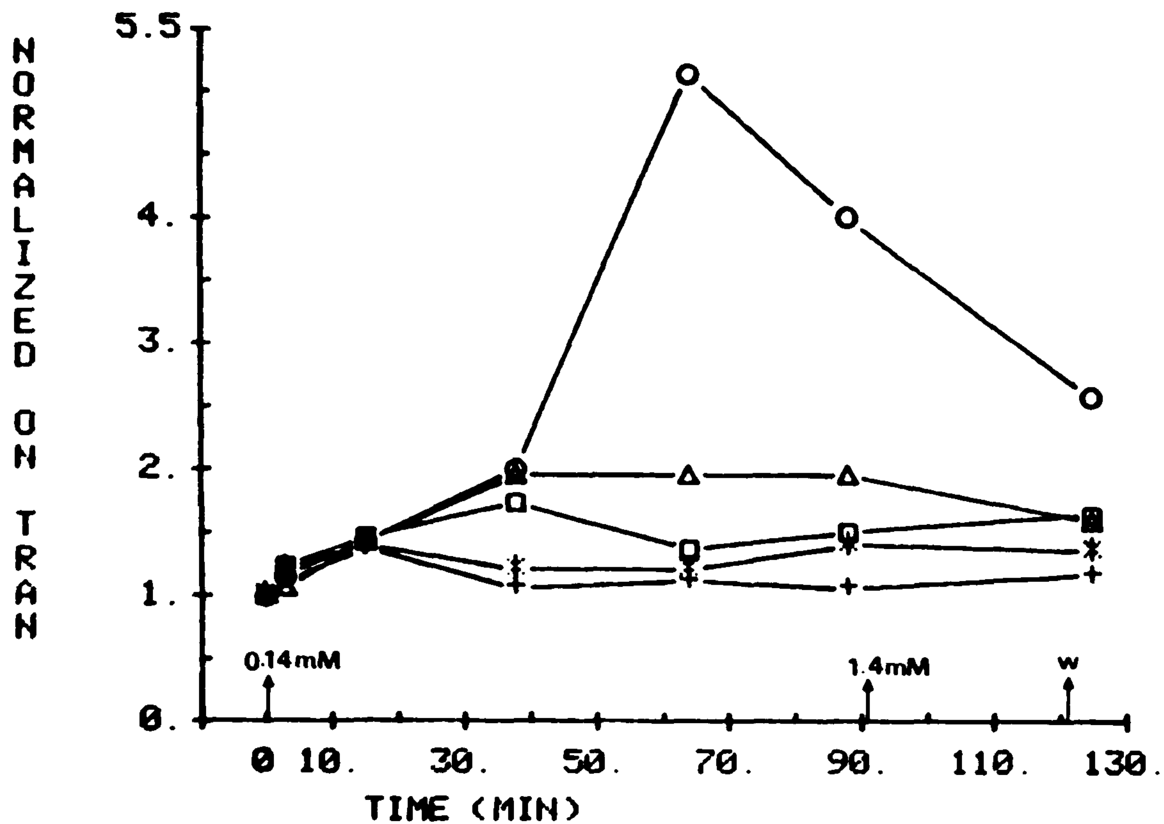
Graph XVI

Curare perfusion experiment. Time course of relative change in ON-transient (see B in figure 83) during 2 consecutive applications of curare (0.14 mM and 1.4 mM). The normalized ON-transient response of the L-neuron is plotted as a function of time. Each curve represents an intensity-response series of the normalized ON-transient response recorded at various times during drug perfusion. The responses were normalized by dividing the responses plotted in each curve (in mv) by the amplitude of the response at time 0 (in mv), before curare perfusion was initiated. This data was obtained from the experiment shown in figure 48. The relative increase in the amplitude of the ON-transient during perfusion with 0.14 mM curare seemed to vary inversely with the intensity of illumination. The largest change was seen in response to Log I = -4 test flashes (compare this graph with Graphs IX, XIII, and XV). The amplitude of the ON-transient in response to dimmer test flashes started to decrease after the application of the more concentrated solution (1.4 mM).

Ordinate: Normalized ON-transient response of L-neuron. Each curve represents an intensity-response series recorded at various times during drug perfusion.

Abscissa: Time course (in minutes) of curare perfusion experiment. Perfusion and wash times are indicated by arrows.

CURARE TO L-NEURON



-o- I =-4
-△- I= -3
-□- I= -2
-✱- I=-1
-+- I= Ø

Graph XVI

Graph XVII

Effect of 0.14 mM curare on response of L-neuron. This data was obtained from the experiment shown in figure 49. The L-neuron potential is plotted as a function of perfusion time. The 3 curves represent the ON-transient response (millivolts with respect to pre-flash dark potential), sustained response (millivolts with respect to pre-flash dark potential) and dark potential (millivolts with respect to dark "resting" potential prior to curare perfusion). During the application of 0.14 mM curare the ON-transient increased in amplitude, the sustained response to light was eliminated (reached the level of the pre-flash dark potential) and the dark potential showed a 2 phased change; first depolarizing, then repolarizing (see figure 49).

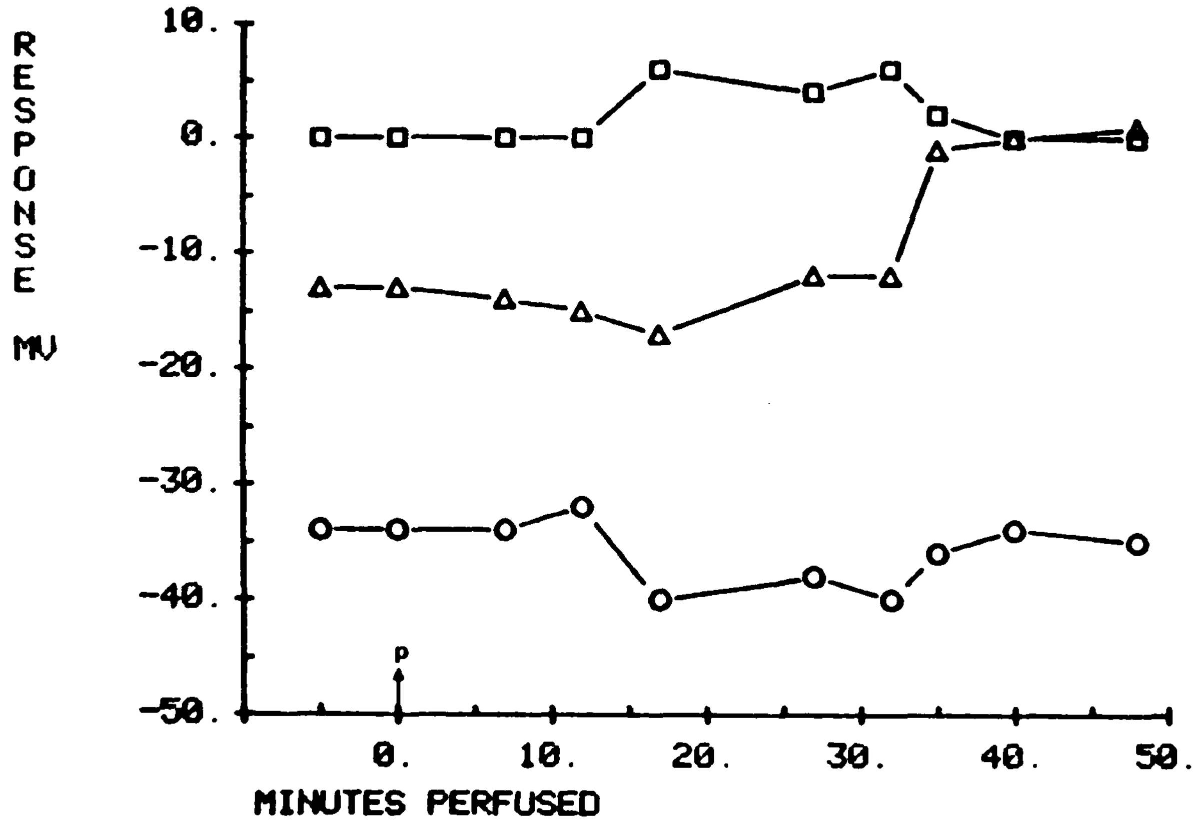
Stimulus intensity: Log I = -2.

Ordinate: L-neuron response (millivolts). The dark potential prior to curare perfusion corresponds to 0 millivolts on the ordinate scale.

Abscissa: Perfusion time (minutes). This cell was lost after 48 minutes perfusion, before Ringer wash was begun. The arrow indicates the onset of curare application.

(See note on pp. 378.)

CURARE (0.14 MM) TO L-NEURON



-O- ON TRANSIENT (MV)
-Δ- SUSTAINED RESP (MV WRT DARK)
-□- DARK POTENTIAL (MV WRT REST)

Graph XVII

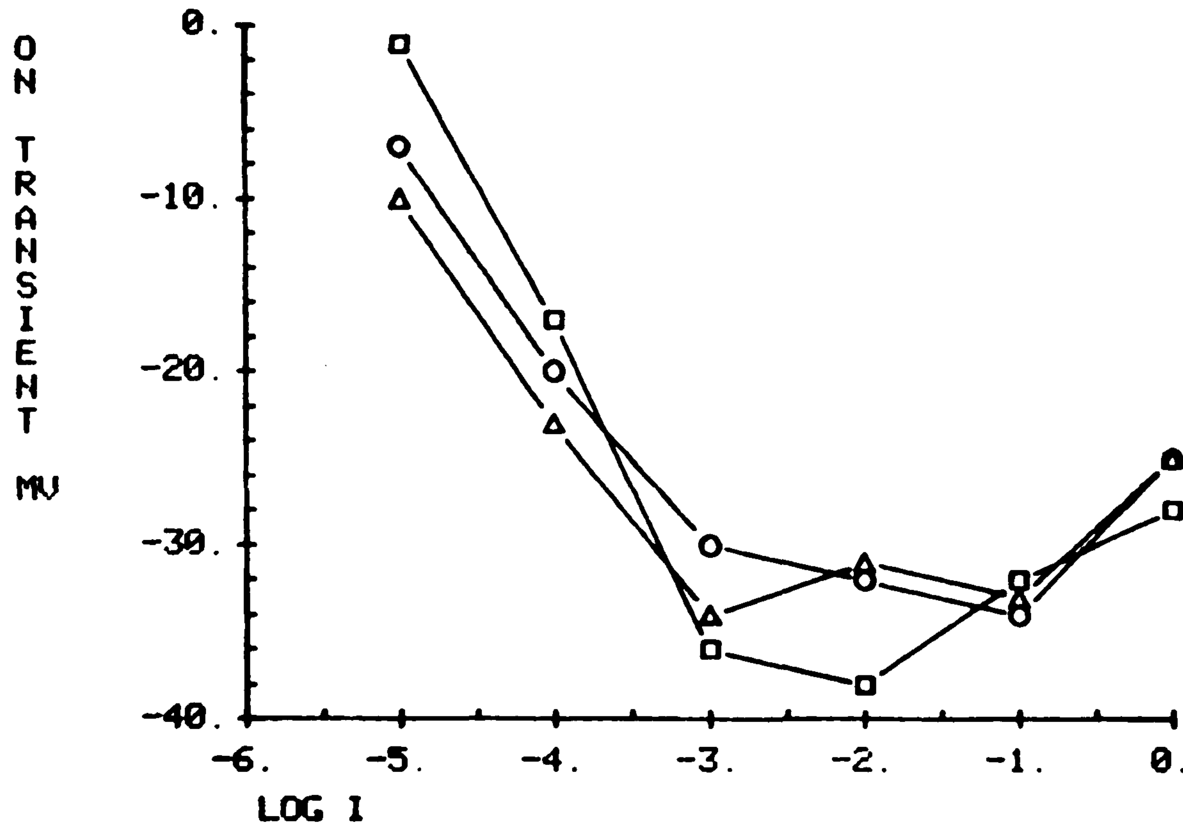
Graph XVIII

Effect of 0.14 mM curare on ON-transient response (see B in figure 83) of L-neuron. The amplitude of the response (mv) is plotted as a function of stimulus intensity (Log I). The 3 curves represent the ON-transient before and during the application of curare (perfused 5 min and 39 min respectively). This data was obtained from the experiment shown in figure 49. During the application of curare the ON-transient increased in amplitude, especially in response to moderate intensity test flashes (see also Graphs XIII, XIV, XV, and XVI).

Ordinate: Amplitude of ON-transient response of L-neuron measured with respect to the dark potential. The dark potential corresponds to 0 mv on the ordinate scale.

Abscissa: Intensity of illumination (Log I).

CURARE TO L-NEURON



-o- ON TRAN (BEFORE CURARE)
-Δ- ON TRAN (CURARE X 5 MIN)
-□- ON TRAN (CURARE X 39 MIN)

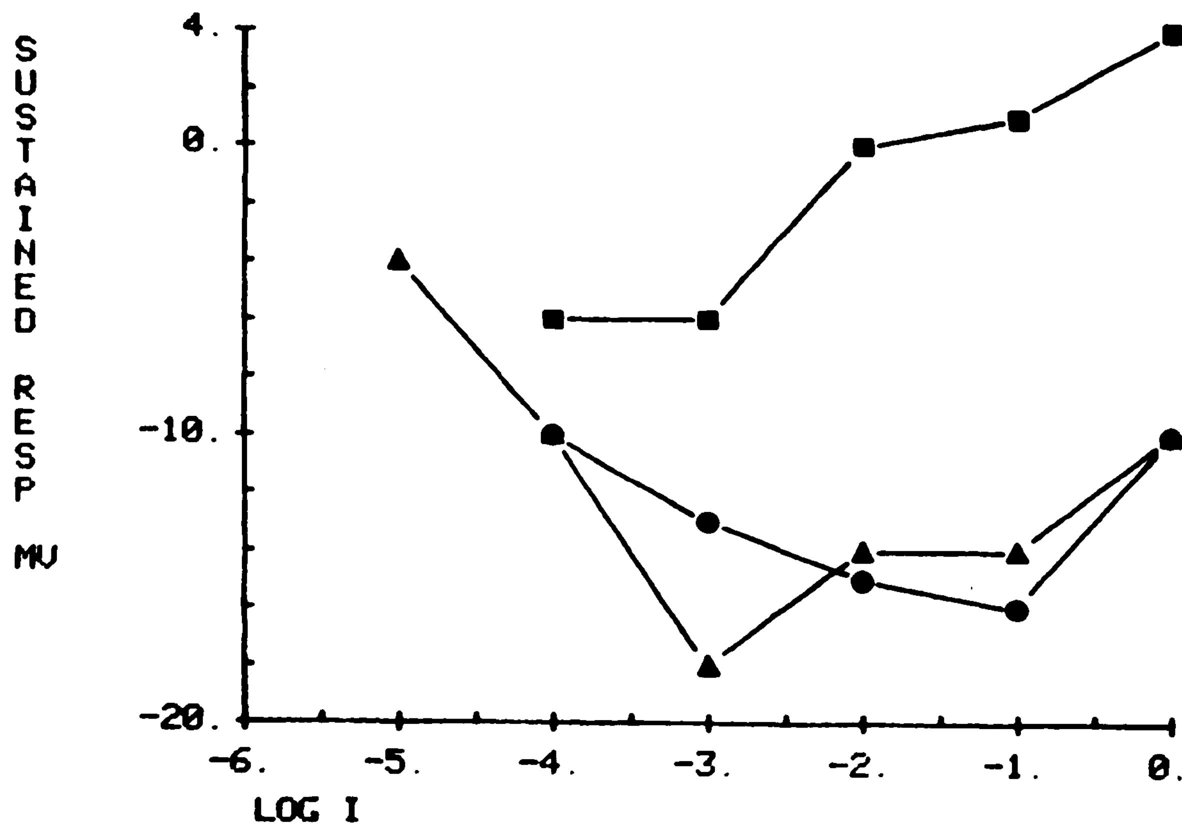
Graph XVIII

Graph XIX

Effect of 0.14 mM curare on sustained component (see D in figure 83) of L-neuron response. The amplitude of the sustained L-neuron response is plotted as a function of stimulus intensity (Log I). This data was obtained from the experiment shown in figure 49. The 3 curves represent the sustained L-neuron response before curare perfusion and at 5 minutes and 39 minutes perfusion respectively. Continued application of curare eliminated the sustained response of the L-neuron, although the ON-transient component of the response was not blocked (see Graph XVIII). The sustained response became smaller during brighter test flashes as perfusion continued.

Ordinate: Amplitude of sustained L-neuron response (mv) measured with respect to the dark potential. The dark potential corresponds to 0 mv on the ordinate scale.

Abscissa: Intensity of illumination (Log I).



Graph XIX

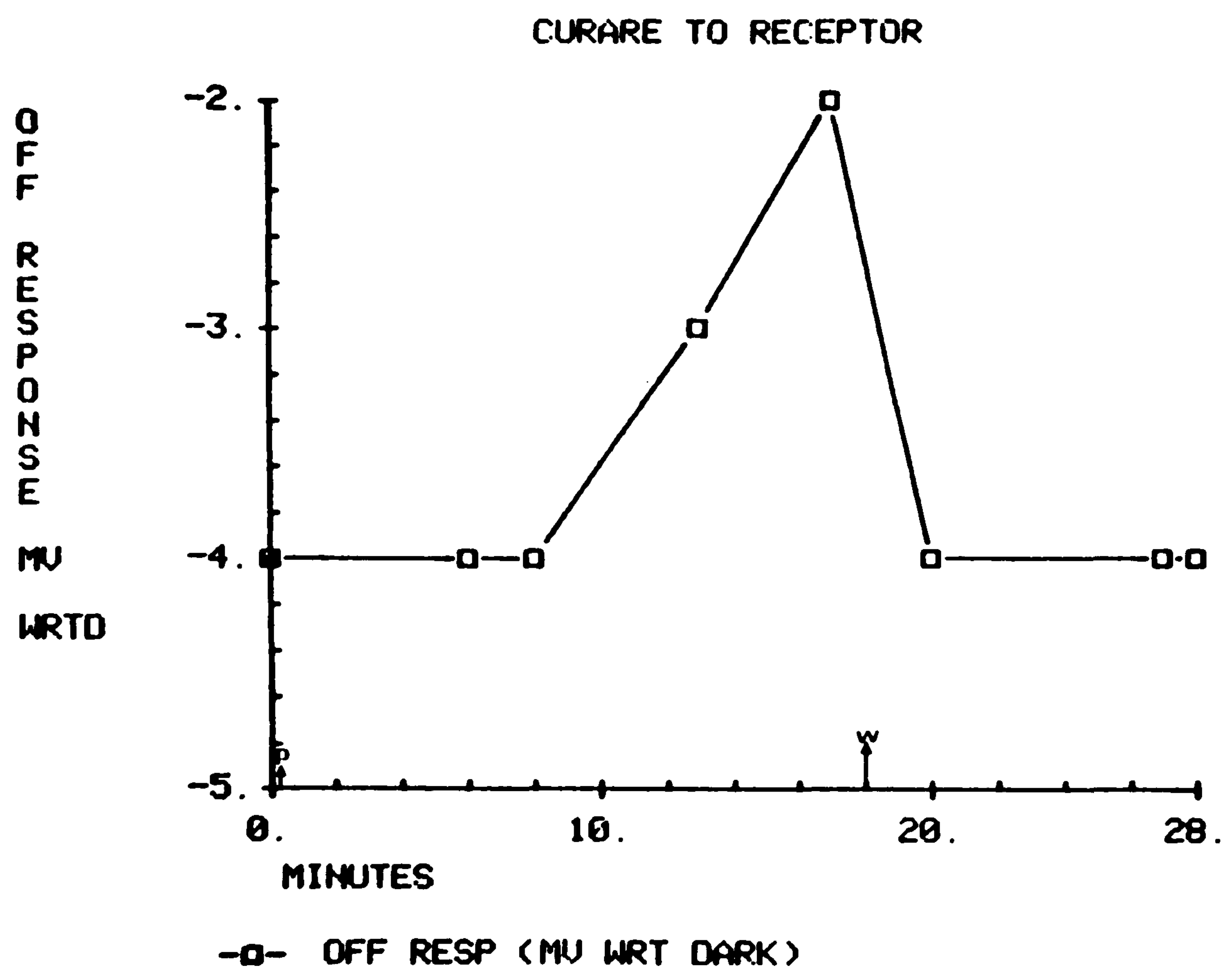
Graph XX

Effect of 1.4 mM curare on the hyperpolarizing OFF response (see Graph II) of the photoreceptor. The amplitude of the OFF response (millivolts with respect to the dark potential) is plotted as a function of time. This data was obtained from the experiment shown in figure 51. During the application of curare the magnitude of the OFF hyperpolarization was reduced.

Stimulus intensity: Log I = -2.

Ordinate: Amplitude of OFF hyperpolarization measured with respect to the dark potential ("mv wrt dark"). The dark "resting" potential is set at 0 millivolts, but is not shown on the ordinate scale in this graph.

Abscissa: Time course of curare perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XX

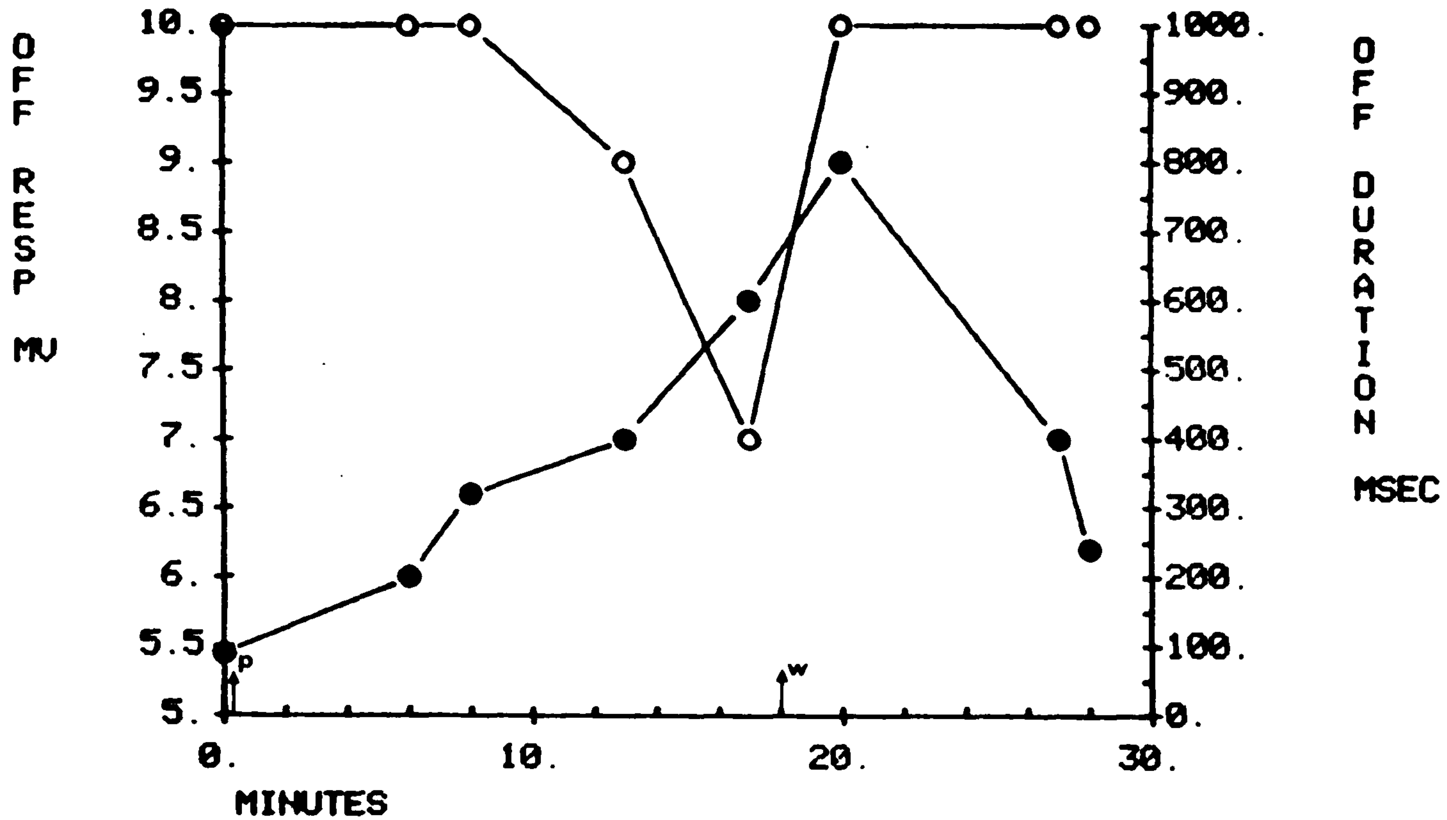
Graph XXI

Effect of 1.4 mM curare on the photoreceptor OFF response from intact nerve preparation. The amplitude (measured from the sustained portion of the light response; see Graph I) and duration of the OFF-transient are plotted as a function of time. This data was obtained from the experiment shown in figure 51. During the application of curare the amplitude of the OFF response was reduced (Left-Y-axis) and the duration of the OFF response increased (Right-Y-axis). The two curves represent the change in the amplitude and duration of the OFF response during the time course of the experiment.

Stimulus intensity: Log I = -2.

Ordinate: (Left axis) Amplitude of OFF-transient measured from the sustained portion of the light response ("mv from sust"). (Right axis) Duration of OFF response in milliseconds.

Abscissa: Time course of curare perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XXI

Graph XXII

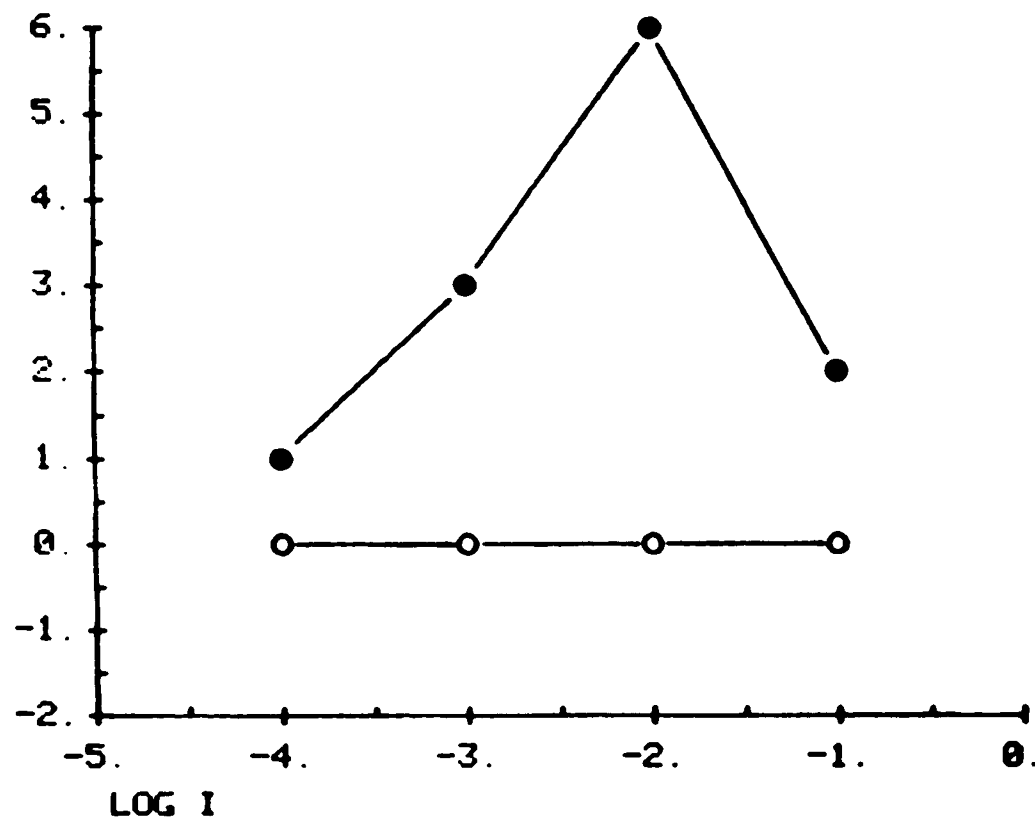
Effect of 0.5 mM curare on receptor OFF response from cut nerve preparation. The amplitude of the OFF response is plotted as a function of stimulus intensity (Log I). The two curves represent the receptor OFF response before curare perfusion and 5 minutes after the application of 0.5 mM curare. This data was obtained from the experiment shown in figure 52. For the purposes of this graph the depolarizing recovery phase of the receptor OFF response was measured because the OFF-hyperpolarization did not undershoot the dark potential (see figure 52). The amplitude of the OFF-transient measured from the sustained portion of the light response did not vary significantly in this experiment. However, as shown in figure 52, the OFF response appeared to be blocked after the application of curare.

Ordinate: Amplitude of receptor OFF response (mv). The repolarizing (recovery) phase of the OFF-response was measured (with respect to the dark potential). The dark potential corresponds to 0 mv on the ordinate scale.

Abscissa: Intensity of illumination (Log I).

CURARE TO RECEPTOR

OFF RESP MV (REPOLARIZATION)



—●— OFF RESP (BEFORE CURARE)
—○— OFF RESP (CURARE X 6 MIN)

Graph XXII

Graph XXIII

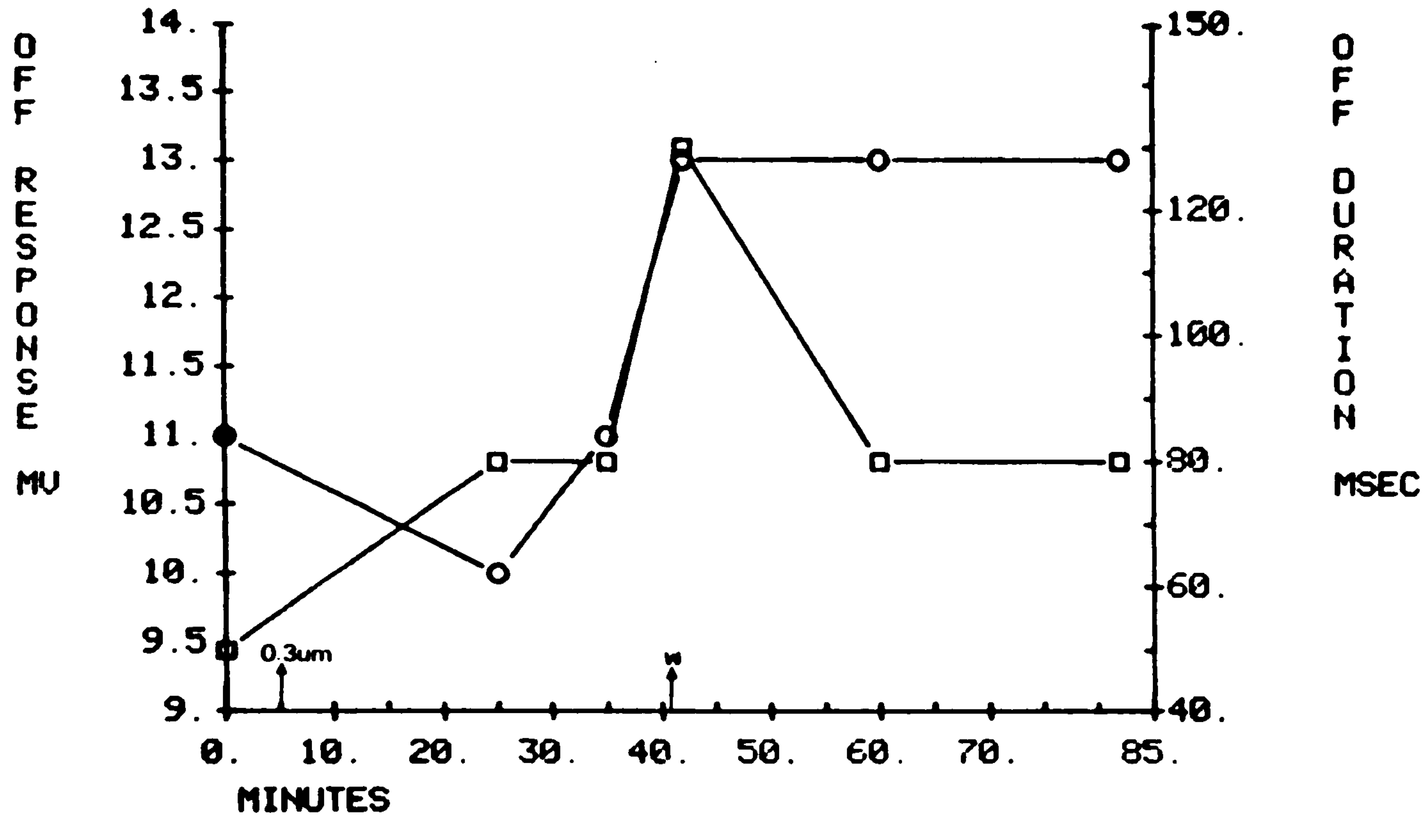
Effect of 0.3 uM eserine on receptor OFF response. The amplitude (Left-Y-axis) and duration (Right-Y-axis) of the receptor OFF response are plotted as a function of time. This data was obtained from the experiment shown in figure 54. During the application of eserine the amplitude of the OFF response showed a 2 phased change, first decreasing in amplitude, then increasing in amplitude. The duration of the OFF response increased and then decreased during the wash period.

Stimulus intensity: Log I = -1.

Ordinate: (Left-Y-axis) Amplitude of receptor OFF-transient (mv) measured from the sustained portion of the response ("mv from sust"). (Right-Y-axis) Duration of OFF response (milliseconds).

Abscissa: Time course of eserine perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.

ESERINE TO PHOTORECEPTOR



—o— OFF RESP (MV FROM SUST)
(R) —□— OFF DURATION (MSEC)

Graph XXIII

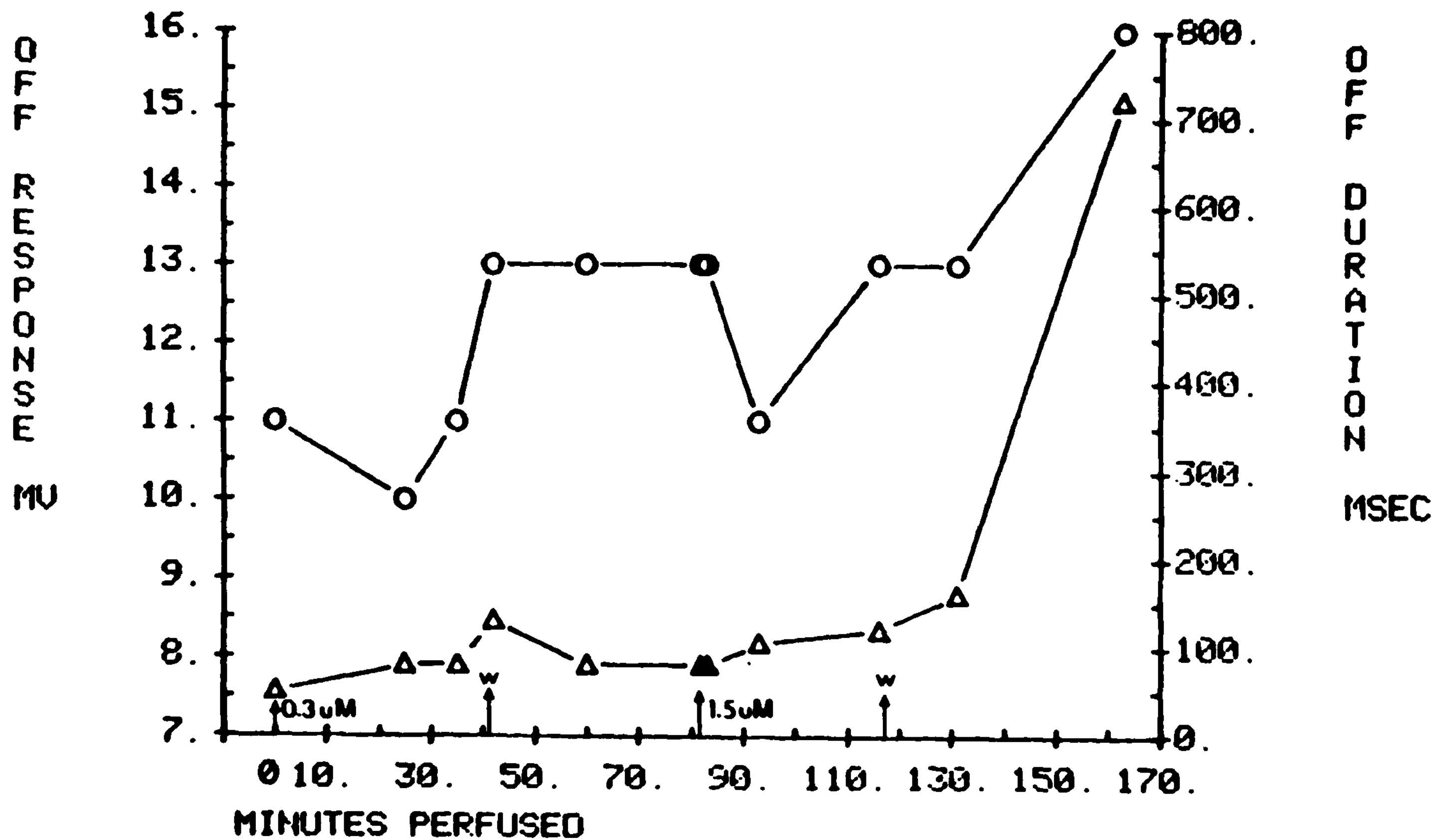
Graph XXIV

Effect of two consecutive eserine perfusions (0.3 uM and 1.5 uM) on receptor OFF response. The amplitude (Left-Y-axis) and duration (Right-Y-axis) are plotted as a function of time. This data was obtained from the experiment shown in figures 54 and 55. Eserine caused a 2 phased change in the amplitude of the receptor OFF-transient which did not reverse following the application of the more concentrated solution (1.5 uM). During eserine perfusion the OFF response increased in duration. The observed increase in duration also did not reverse following perfusion with the higher dose (1.5 uM).

Stimulus intensity: Log I = -1.

Ordinate: (Left-Y-axis) Amplitude of receptor OFF-transient measured from sustained portion of receptor light response ("mv from sust"). (Right-Y-axis) Duration of receptor OFF response (msec).

Abscissa: Time course of eserine perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XXIV

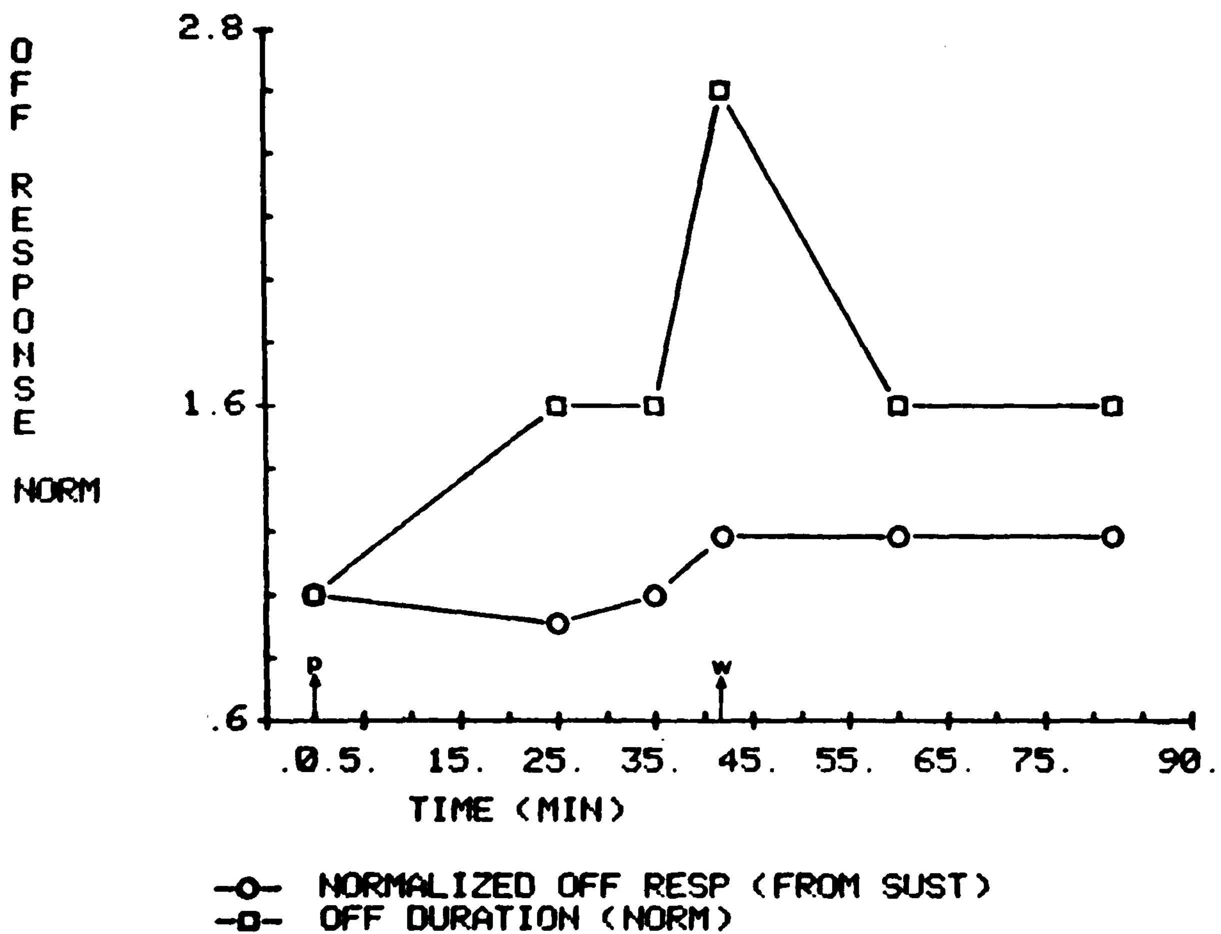
Graph XXV

Effect of 0.3 uM eserine on amplitude and duration of receptor OFF-response. This data was obtained from the experiment shown in figure 54. The normalized responses are plotted as a function of time. The responses were normalized by dividing all responses by the amplitude (millivolts from sustained light response) and duration (milliseconds) of the OFF-response at time 0, before eserine perfusion was initiated. The relative increase in the OFF-response duration was greater than the relative increase in the amplitude of the OFF-transient (see also Graph XXIII).

Stimulus intensity: Log I = -1.

Ordinate: Normalized amplitude and duration of receptor OFF response.

Abscissa: Time course of eserine perfusion experiment. Perfusion and wash times are indicated by arrows.



Graph XXV

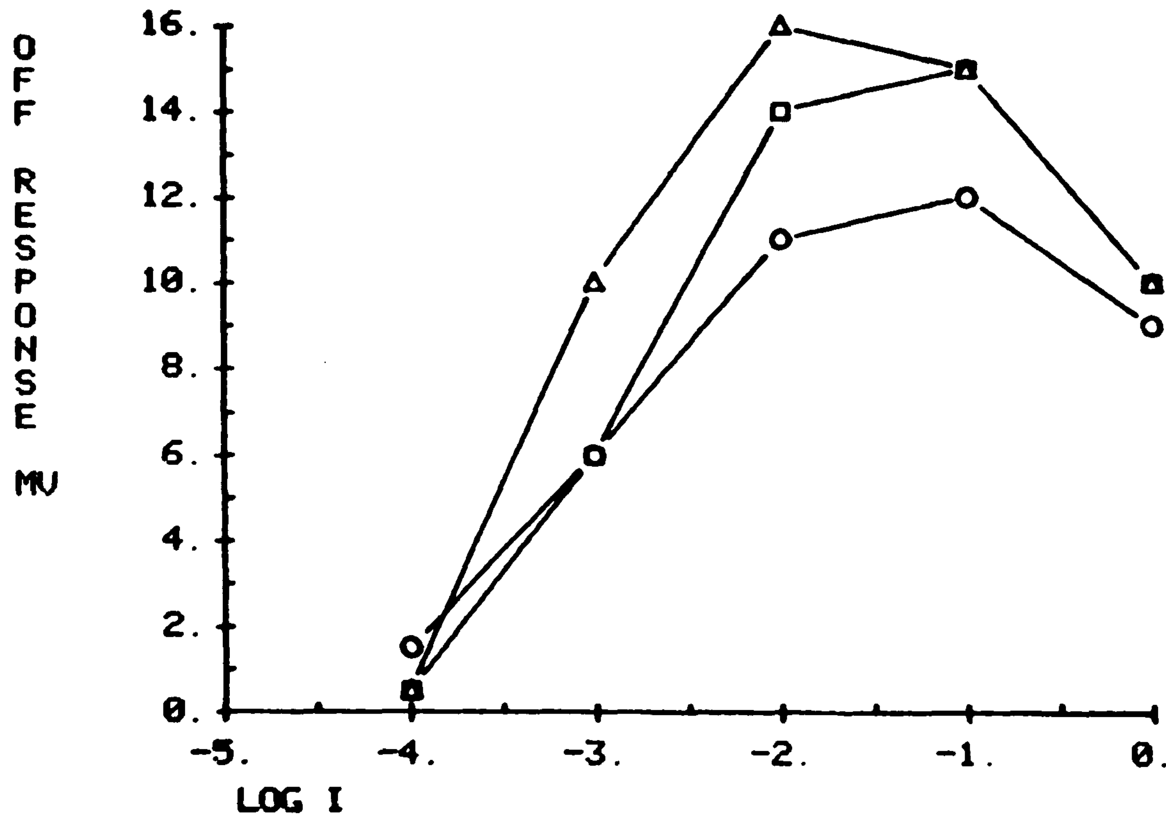
Graph XXVI

Intensity-response series recorded from photoreceptor during 2 consecutive eserine perfusions (0.3 uM and 1.5 uM). The amplitude of the receptor OFF-response is plotted as a function of stimulus intensity (Log I). The 3 curves represent the amplitude of the OFF-response before eserine perfusion and after the application of 0.3 uM (perfused 43 min) and 1.5 uM (perfused 25 min) eserine. This data was obtained from the experiment shown in figures 54 and 55. The amplitude of the receptor OFF-transient (measured from the sustained portion of the light response) increased during the application of eserine. This increase in amplitude was more pronounced following less intense test flashes.

Ordinate: Amplitude of receptor OFF-response (mv) measured from the sustained portion of the light response.

Abscissa: Intensity of illumination (Log I).

ESERINE TO PHOTORECEPTOR



-□- 0.3 UM ESERINE X 43MIN
-△- 1.5 UM ESERINE X 25 MIN
-○- BEFORE ESERINE

Graph XXVI

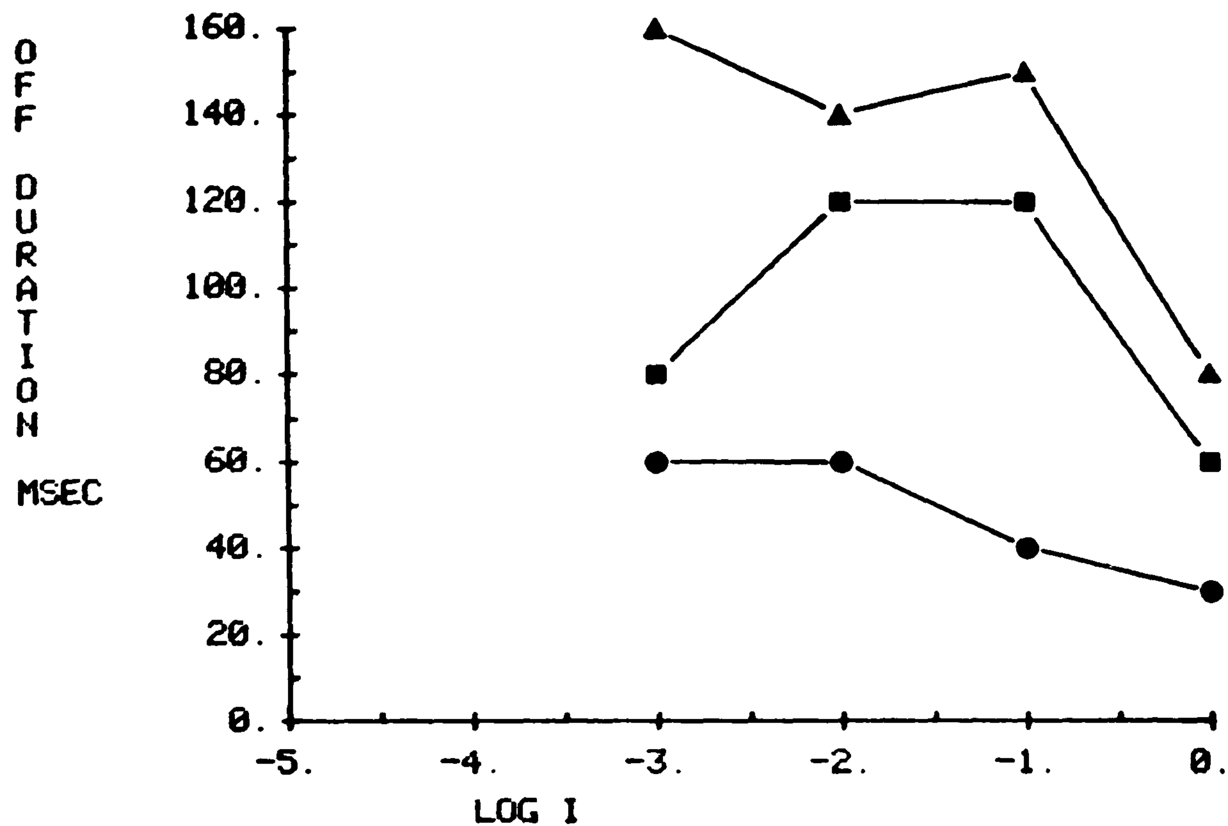
Graph XXVII

Intensity-response series recorded from photoreceptor during two consecutive eserine perfusions (0.3 uM and 1.5 uM). The duration of the receptor OFF-transient (milliseconds) is plotted as a function of stimulus intensity (Log I). The 3 curves represent the OFF-response duration before eserine perfusion and after the application of 0.3 uM (perfused 43 minutes) and 1.5 uM (perfused 25 minutes) eserine. This data was obtained from the experiment shown in figures 54 and 55. The duration of the receptor OFF transient (milliseconds) increased during eserine perfusion.

Ordinate: Duration of receptor OFF-response (msec).

Abscissa: Intensity of illumination (Log I).

ESERINE TO PHOTORECEPTOR



—●— BEFORE ESERINE
—■— 0.3 UM ESERINE X 43 MIN
—▲— 1.5 UM ESERINE X 25 MIN

Graph XXVII

Graph XXVIII

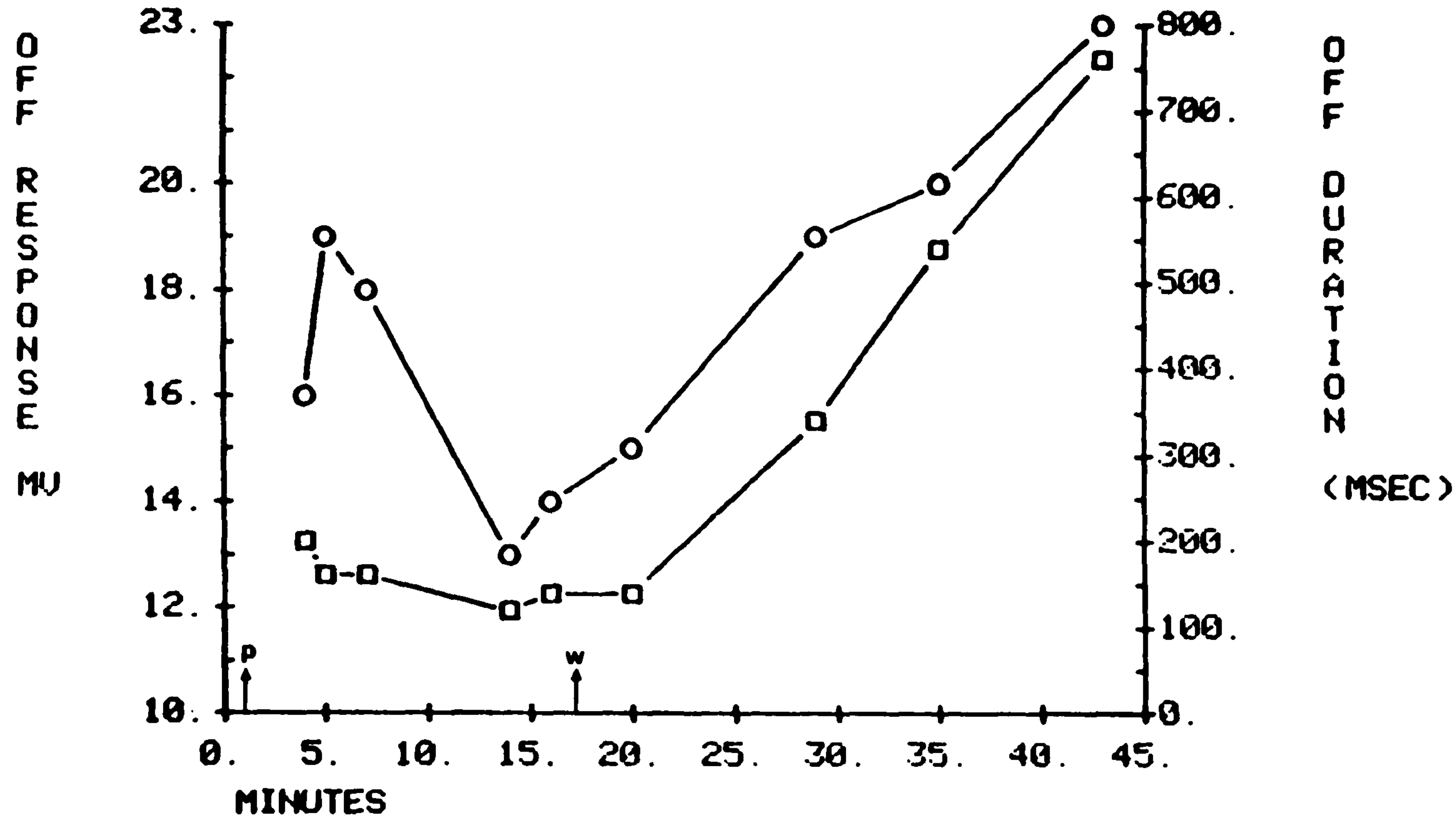
Effect of 1.5 uM eserine on the amplitude and duration of the receptor OFF-response. The amplitude (Left-Y-axis) and duration (Right-Y-axis) are plotted as a function of time. This data was obtained from the experiment shown in figure 58. This preparation had developed bistable behavior after prior treatment with curare (see figure 57). Eserine had a 2 phased effect on the OFF-response; the amplitude and duration of the OFF-transient first decreased, then increased (see figure 58).

Stimulus intensity: Log I = -2.

Ordinate: (Left-Y-axis) Amplitude of OFF-transient measured from sustained portion of light response ("mv from sust"). (Right-Y-axis) Duration of OFF-response (milliseconds).

Abscissa: Time course of eserine perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.

ESERINE TO RECEPTOR



-o- OFF RESP (MV FROM SUST)
(R) -□- OFF DURATION (MSEC)

Graph XXVIII

Graph XXIX

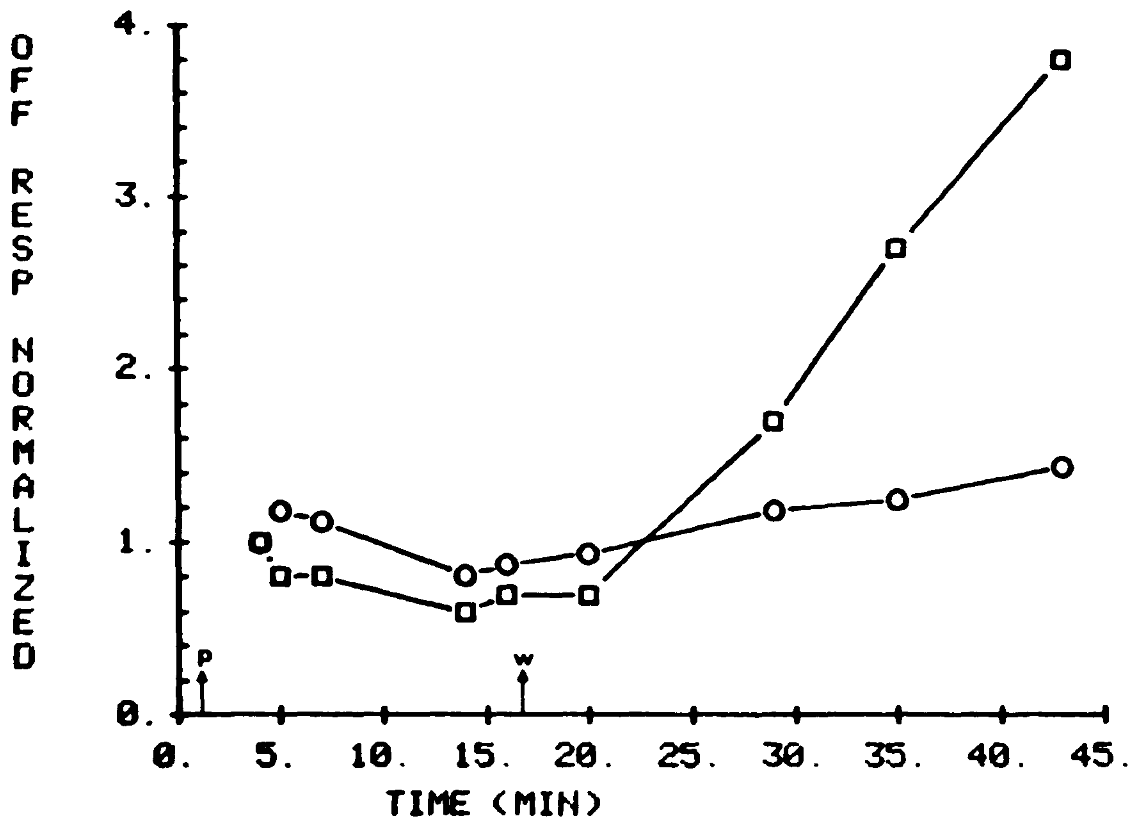
Effect of 1.5 uM eserine on the amplitude and duration of the receptor OFF-response. This data was obtained from the experiment shown in figure 58. The normalized amplitude and duration of the receptor OFF-response are plotted as a function of time. The data was normalized by dividing the value of all responses by their value (millivolts and milliseconds respectively) before eserine perfusion was initiated. During the wash period the OFF-response duration increased to a greater extent than the amplitude of the OFF-transient (see Graph XXVIII and figure 58).

Stimulus intensity: Log I = -2.

Ordinate: Normalized OFF-response. The amplitude of the OFF-transient was measured from the sustained portion of the light response; the OFF duration was in milliseconds.

Abscissa: Time course of eserine perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.

ESERINE TO RECEPTOR



—o— NORALIZED OFF RESP (FROM SUST)
—□— NORMALIZED OFF RESP (DUR)

Graph XXIX

Graph XXX

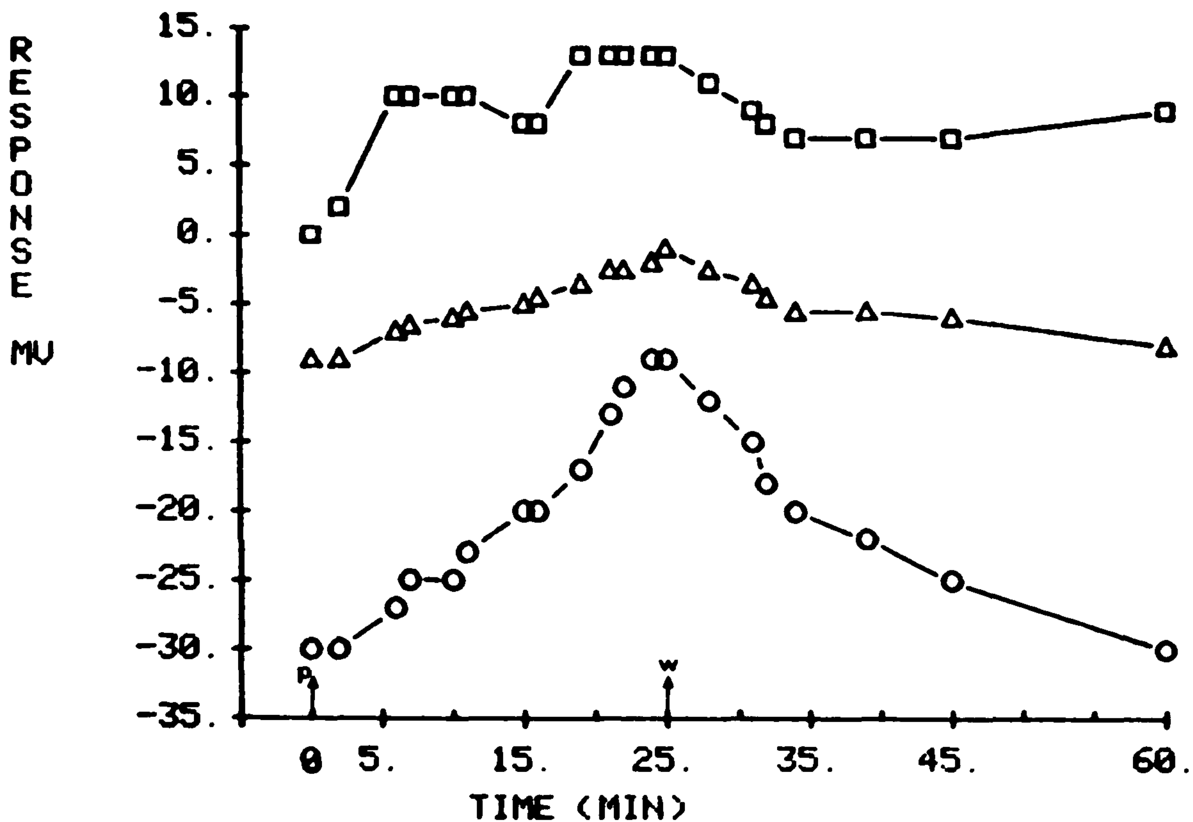
Effect of 10 mM carbachol on the response of the L-neuron. The dark potential (see A in figure 83), ON-transient (see B in figure 83) and sustained (see D in figure 83) phase of the L-neuron response are plotted as a function of time. This data was obtained from the experiment shown in figure 63. The 3 curves represent the change in dark potential (with respect to the dark "resting" potential before carbachol perfusion), the hyperpolarizing ON-transient (millivolts with respect to the dark potential prior to light-ON), and the sustained response (millivolts with respect to the dark potential prior to light-ON) of the L-neuron. During the application of carbachol the dark potential depolarized and the amplitude of the ON-transient and sustained light response were reduced.

Stimulus intensity: Log I = -3.

Ordinate: Amplitude of L-neuron response (mv). The dark potential corresponds to 0 millivolts on the ordinate scale.

Abscissa: Time course of carbachol perfusion experiment. Perfusion and wash times are indicated by arrows.

(See note on pp. 378.)



Graph XXX

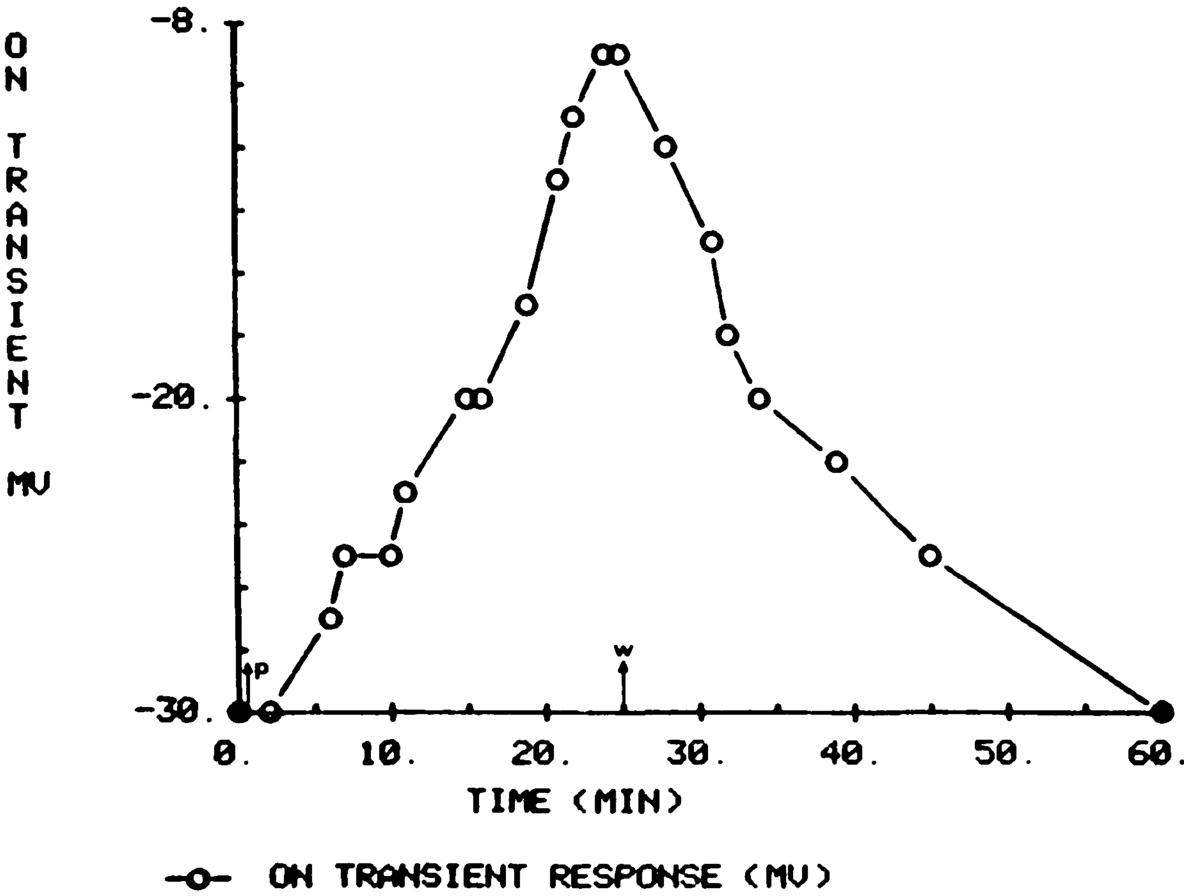
Graph XXXI

Effect of 10 mM carbachol on ON-transient phase (see B in figure 83) of L-neuron response. The amplitude of the ON-transient (mv with respect to dark potential) is plotted as a function of time. This data was obtained from the experiment shown in figure 63. During carbachol perfusion the amplitude of the ON-transient decreased from -30 mv to -8 mv below the pre-flash dark potential.

Stimulus intensity: Log I = -3.

Ordinate: Amplitude of ON-transient response of L-neuron with respect to the pre-flash dark potential.

Abscissa: Time course of carbachol perfusion experiment. Perfusion and wash times are indicated by arrows.



Graph XXXI

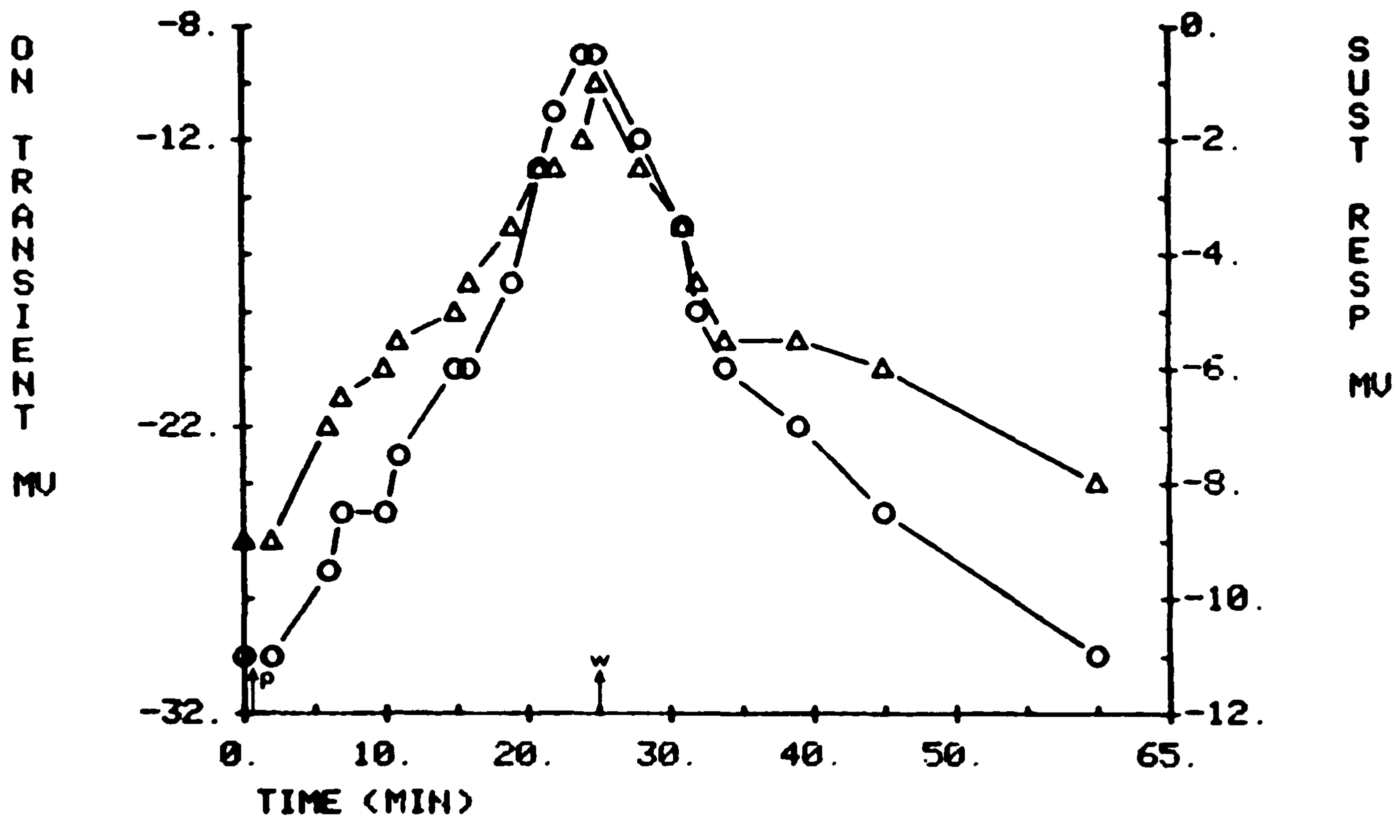
Graph XXXII

Effect of 10 mM carbachol on ON-transient and sustained components of L-neuron response (see Graphs XXX and XXXI). The amplitude of the ON-transient (Left-Y-axis) and sustained response (Right-Y-axis) are plotted as a function of time. This data was obtained from the experiment shown in figure 63. During the application of carbachol the relative decrease in the amplitude of ON-transient and sustained response were similar. The amplitude of each response (in millivolts) was measured with respect to the pre-flash dark potential. Note that the sustained response was almost eliminated (reached 0 mv). However, the pre-flash dark potential had depolarized to a more positive level than before perfusion (see Graph XXX).

Stimulus intensity: Log I = -3.

Ordinate: (Left-Y-axis) Amplitude of ON-transient response (mv with respect to pre-flash dark potential. (Right-Y-axis) Amplitude of sustained L-neuron response (mv with respect to pre-flash dark potential). The pre-flash dark potential, which corresponds to 0 mv on the ordinate scale, was actually more positive than before the application of carbachol. The peak portions of the response around the time of washing were matched arbitrarily for purposes of comparison.

Abscissa: Time course of carbachol perfusion experiment. Perfusion and wash times are indicated by arrows.



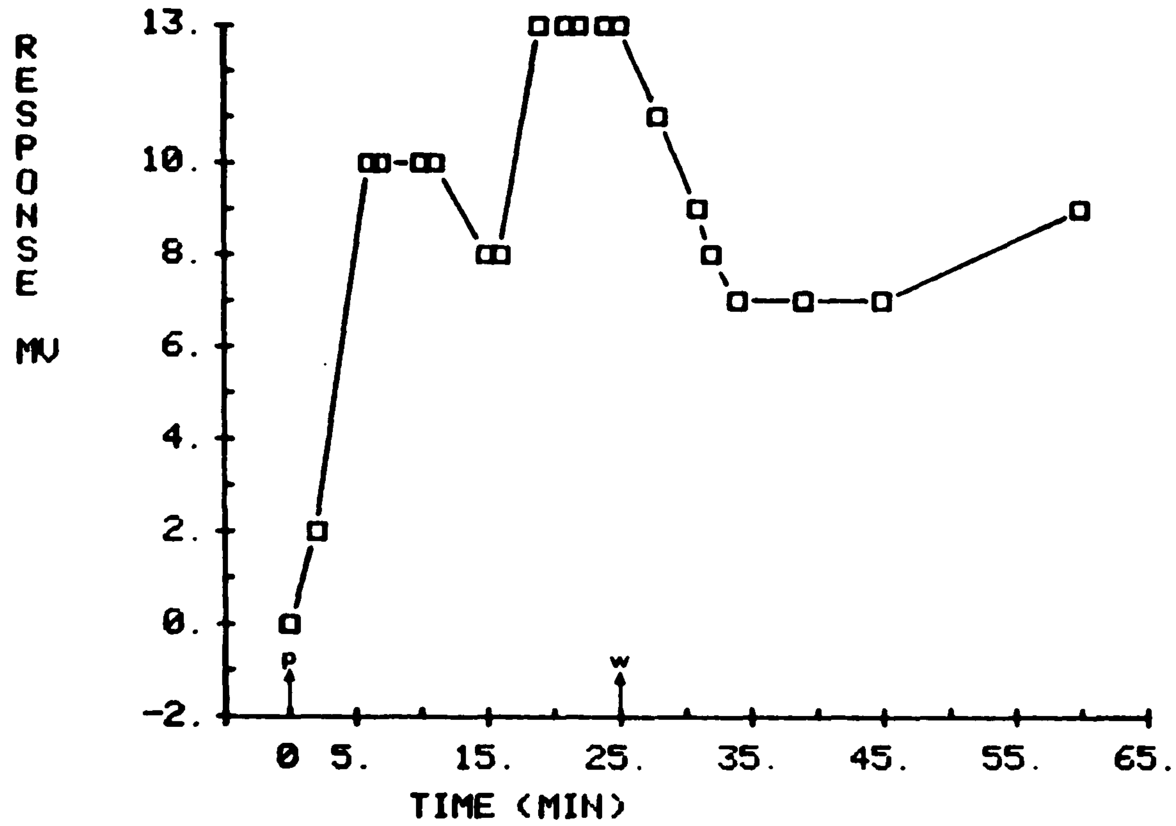
Graph XXXII

Graph XXXIII

Effect of 10 mM carbachol on dark potential (see A in figure 83) of L-neuron. The change in dark potential (with respect to the dark "resting" potential prior to carbachol perfusion) is plotted as a function of time. This data was obtained from the experiment shown in figure 63. During the application of carbachol the L-neuron dark potential depolarized and only partially recovered following Ringer wash. However, the waveform of the L-neuron light response recovered almost completely during the wash period (see figure 63).

Ordinate: Change in L-neuron dark potential (millivolts). The dark "resting" potential prior to the application of carbachol corresponds to 0 mv on the ordinate scale.

Abscissa: Time course of carbachol perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XXXIII

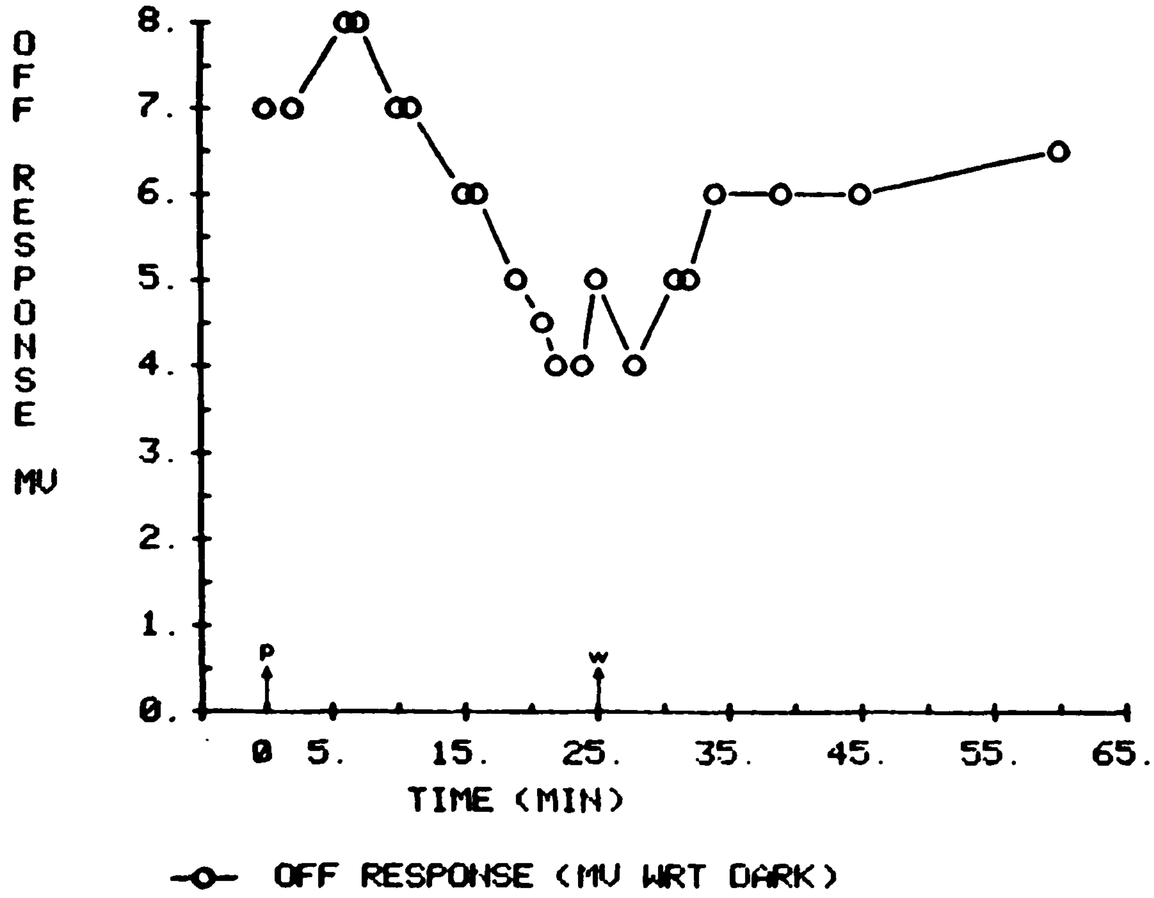
Graph XXXIV

Effect of 10 mM carbachol on depolarizing OFF-transient (see E in figure 83) of L-neuron response. The amplitude of the OFF-depolarization (millivolts with respect to pre-flash dark potential) is plotted as a function of time. This data was obtained from the experiment shown in figure 63. During the application of carbachol the OFF-depolarization transiently increased in amplitude and then decreased. Note the OFF-depolarization was not eliminated (did not reach 0 mv) despite the fact that the dark potential was apparently more depolarized at this time (see Graph XXXIII).

Stimulus intensity: Log I = -3.

Ordinate: Amplitude of depolarizing OFF response (millivolts with respect to the pre-flash dark potential). The dark potential prior to light-ON corresponds to 0 mv on the ordinate scale.

Abscissa: Time course of carbachol perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XXXIV

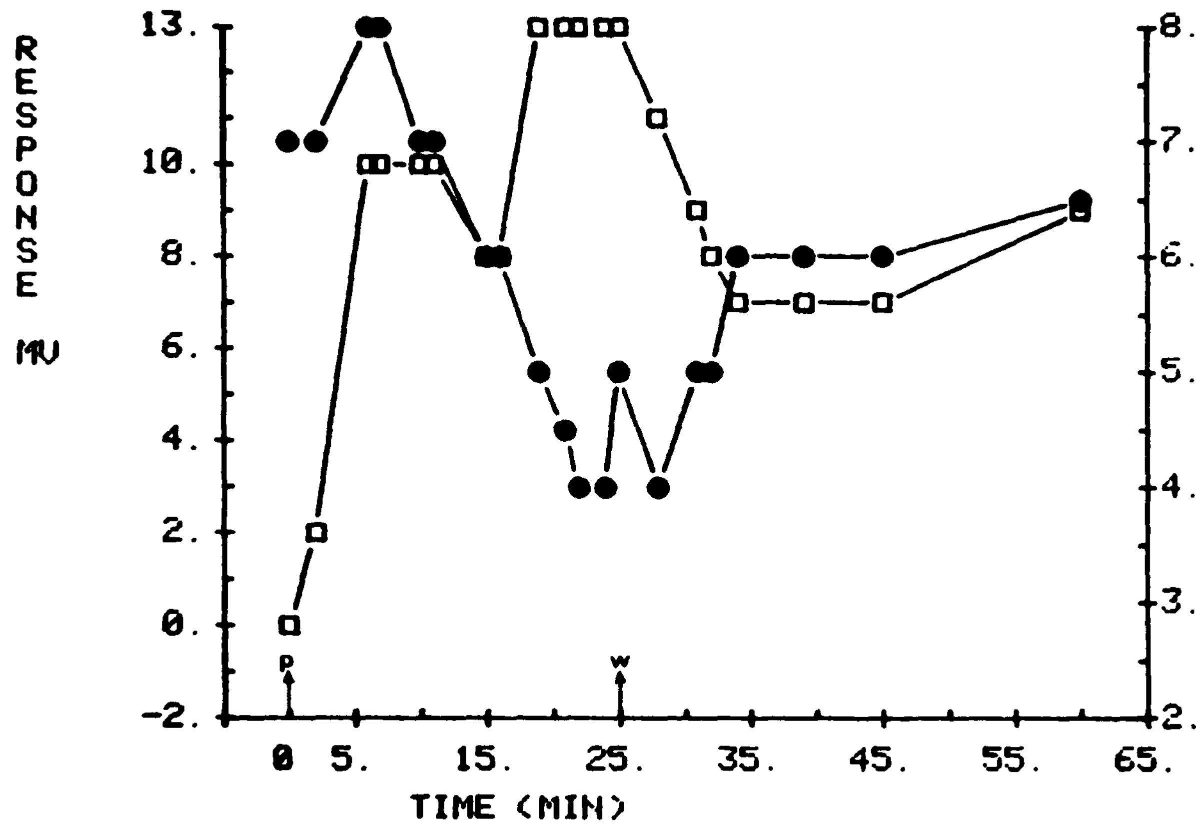
Graph XXXV

Effect of 10 mM carbachol on dark potential and depolarizing OFF-transient of L-neuron response. The dark potential (Left-Y-axis) and OFF response (Right-Y-axis) are plotted as a function of time. This data was obtained from the experiment shown in figure 63. During the application of carbachol the dark potential depolarized (with respect to the dark "resting" potential prior to carbachol perfusion) and the depolarizing OFF-transient was transiently enhanced and then became smaller. Note that the apparent reduction in the amplitude of the depolarizing OFF response (which was measured with respect to the dark potential prior to light-ON) occurred at a time when the L-neuron had apparently depolarized (see Graphs XXX and XXXII). Consequently, the depolarizing OFF response may actually have been enhanced during this period, because it was measured with respect to a dark potential that had already depolarized significantly.

Stimulus intensity: Log I = -3.

Ordinate: (Left-Y-axis) Amplitude of dark potential with respect to the dark "resting" potential ("mv wrt rest") before carbachol perfusion. The dark potential before carbachol perfusion corresponds to 0 mv on the ordinate scale (Left-Y-axis). (Right-Y-axis) Amplitude of depolarizing OFF-transient measured with respect to the pre-flash dark potential just before light-ON ("mv wrt dark"). The OFF response was always detected despite the fact that the dark potential had depolarized.

Abscissa: Time course of carbachol perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XXXV

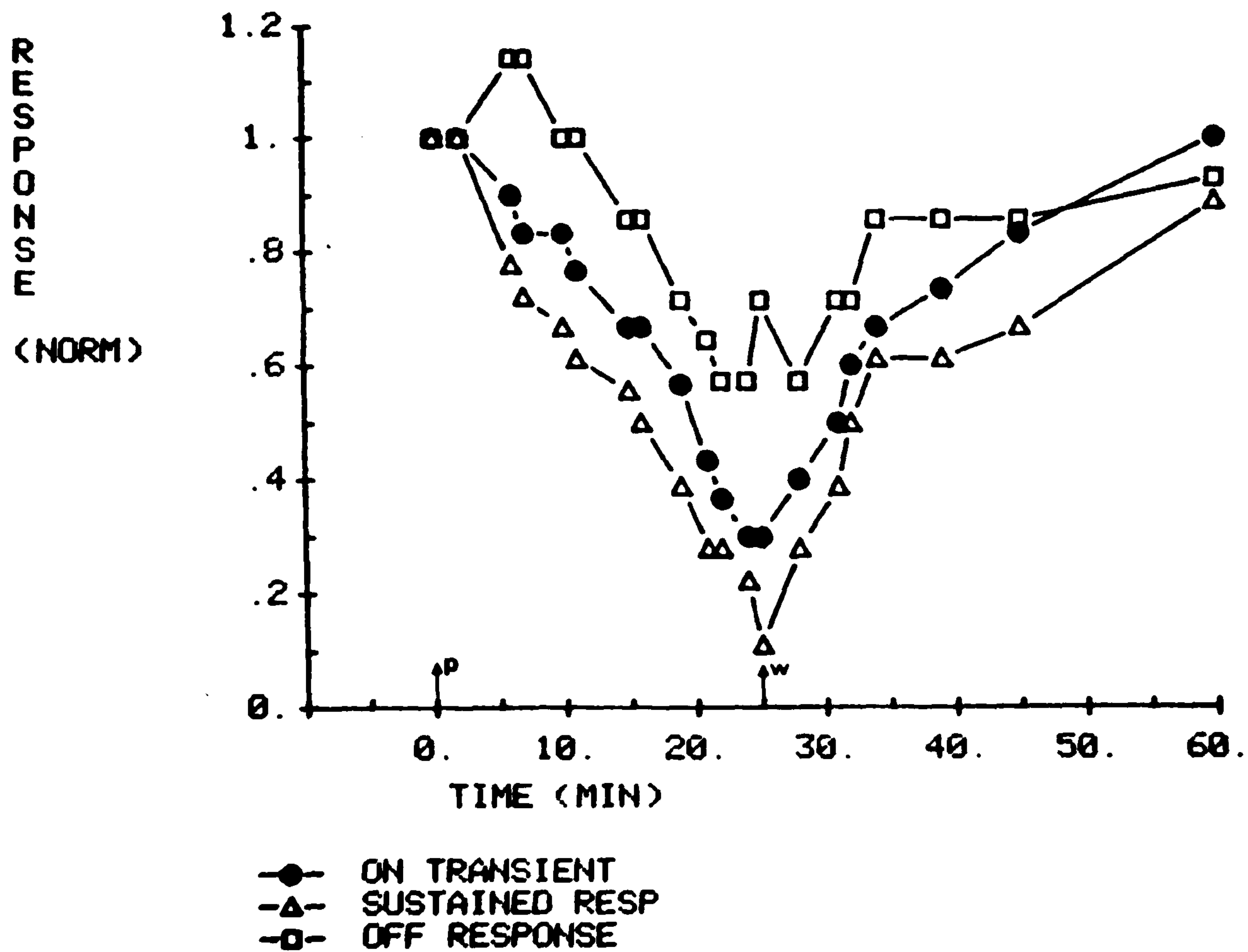
Graph XXXVI

Effect of 10 mM carbachol on response of L-neuron. The normalized responses are plotted as a function of time. The responses were normalized by dividing the amplitude (in mv) at various intervals during the time course of the experiment by the response amplitude at time 0 (before carbachol perfusion was initiated). This data was obtained from the experiment shown in figure 63. The 3 curves represent ON-transient (see B in figure 83), sustained (see D in figure 83) and OFF-transient (see E in figure 83) response of the L-neuron. The responses were measured as described in Graphs XXXI, XXXII, XXXIV, and XXV. During the application of carbachol all 3 components of the L-neuron response were reduced in amplitude. The relative decrease was largest for the sustained response.

Stimulus intensity: Log I = -3.

Ordinate: Normalized L-neuron response (see above discussion).

Abscissa: Time course of carbachol perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XXXVI

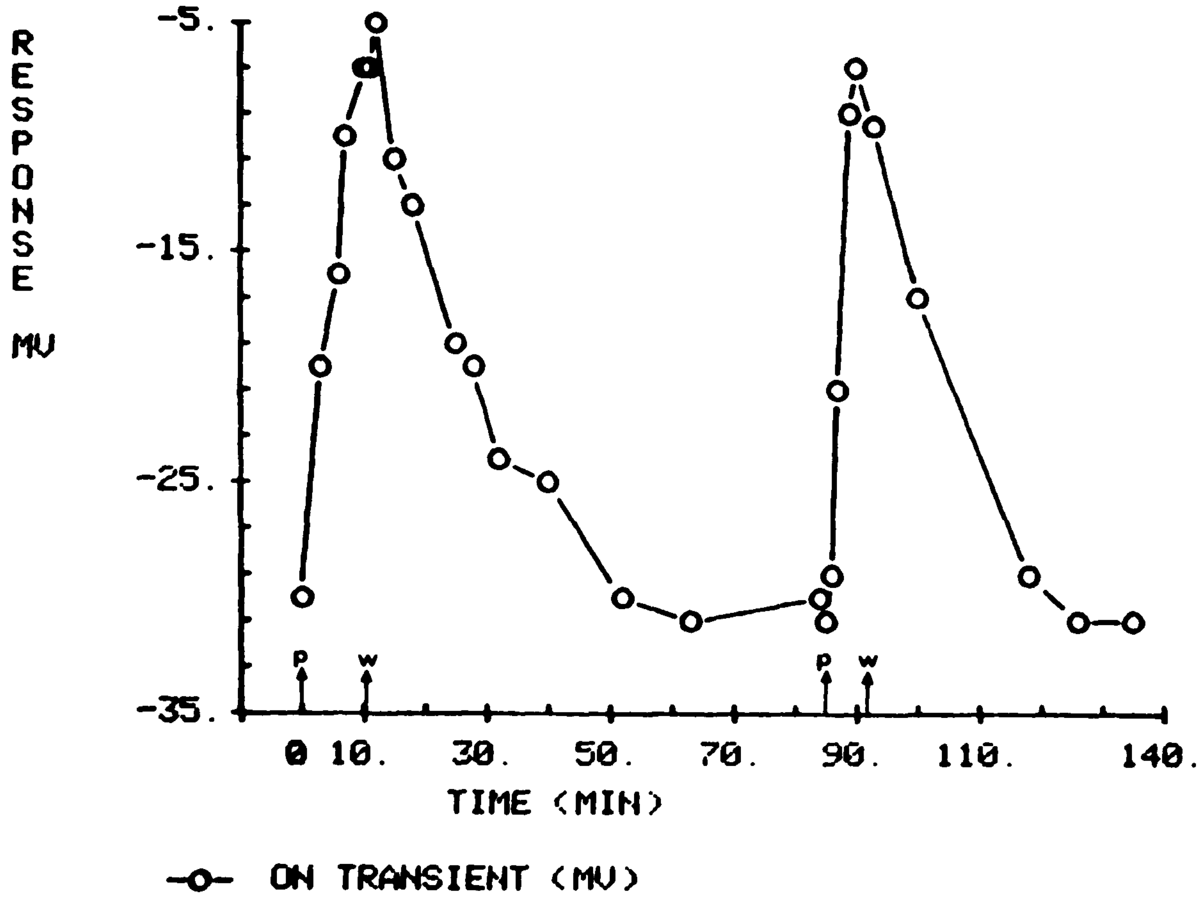
Graph XXXVII

Effect of 2 consecutive applications of 10 mM carbachol on ON-transient response of an L-neuron. The amplitude of the hyper-polarizing ON-transient response to light (see B in figure 83) is plotted as a function of time. This data was obtained from the experiment shown in figures 64 and 65. Carbachol reversibly reduced the amplitude of the ON-transient during two consecutive perfusions and washes.

Stimulus intensity: Log I = -3.

Ordinate: Amplitude of ON-transient response of L-neuron (millivolts with respect to the pre-flash dark potential). The pre-flash dark potential, which corresponds to 0 millivolts on the ordinate scale, is not shown in this graph.

Abscissa: Time course of carbachol perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XXXVII

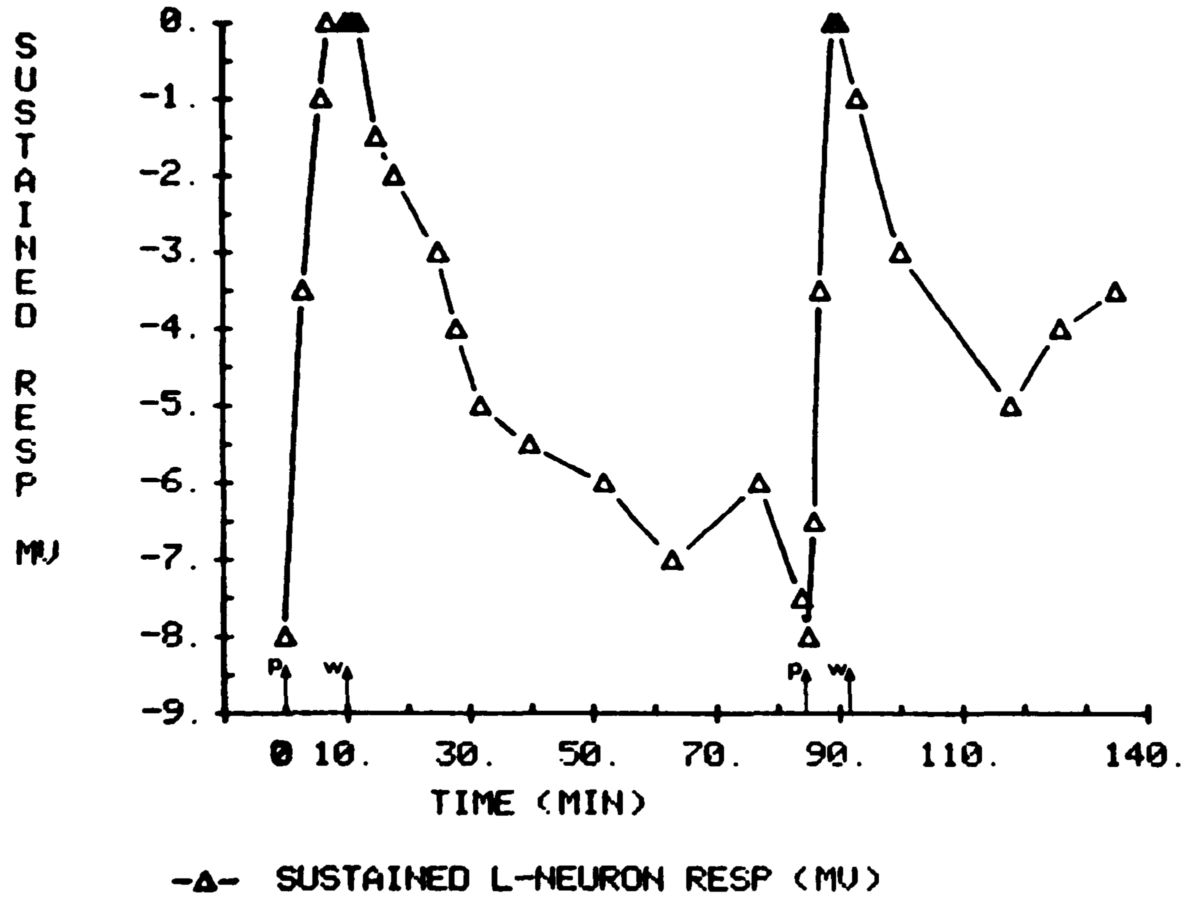
Graph XXXVIII

Effect of 2 consecutive applications of 10 mM carbachol on sustained light response (see D in figure 83) of L-neuron. The amplitude of the sustained component of the light response is plotted as a function of time. This data was obtained from the experiment shown in figures 64 and 65. Carbachol reversibly eliminated the sustained light response during two consecutive perfusions and washes. Note the sustained light response reached 0 millivolts, which is equivalent to the pre-flash dark potential.

Stimulus intensity: Log I = -3.

Ordinate: Amplitude of sustained L-neuron light response (millivolts) which was measured with respect to the dark potential before light-ON. The pre-flash dark potential corresponds to 0 millivolts on the ordinate scale.

Abscissa: Time course of carbachol perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



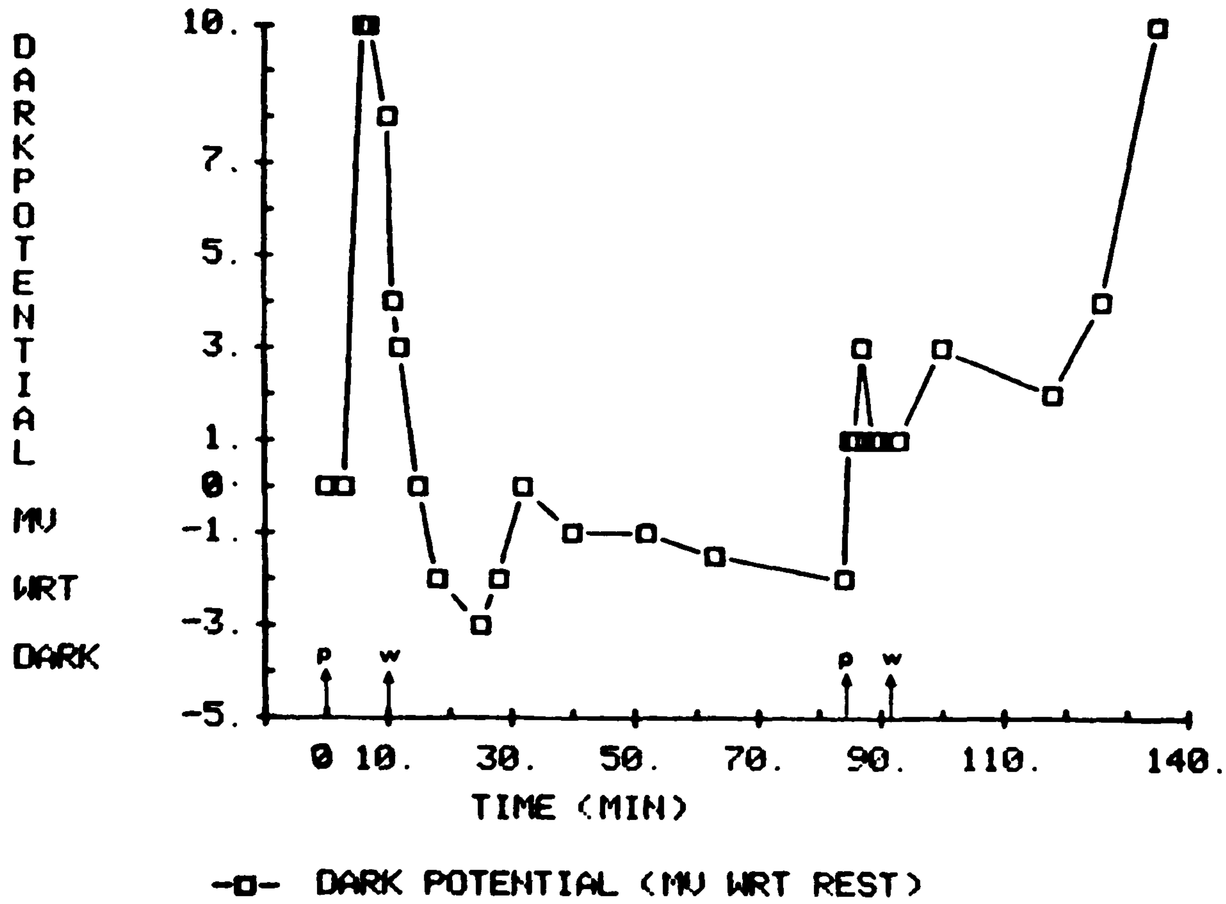
Graph XXXVIII

Graph XXXIX

Effect of 2 consecutive applications of 10 mM carbachol on the dark potential (see A in figure 83) of the L-neuron. The pre-flash dark potential is plotted as a function of time. This data was obtained from the experiment shown in figures 64 and 65. Carbachol depolarized the L-neuron during 2 consecutive perfusions and washes. During the wash period the dark potential repolarized to a more negative level than before the application of carbachol.

Ordinate: L-neuron dark potential (millivolts with respect to dark potential before carbachol perfusion). The dark "resting" potential before carbachol treatment corresponds to 0 millivolts on the ordinate scale.

Abscissa: Time course of carbachol perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XXXIX

Graph XL

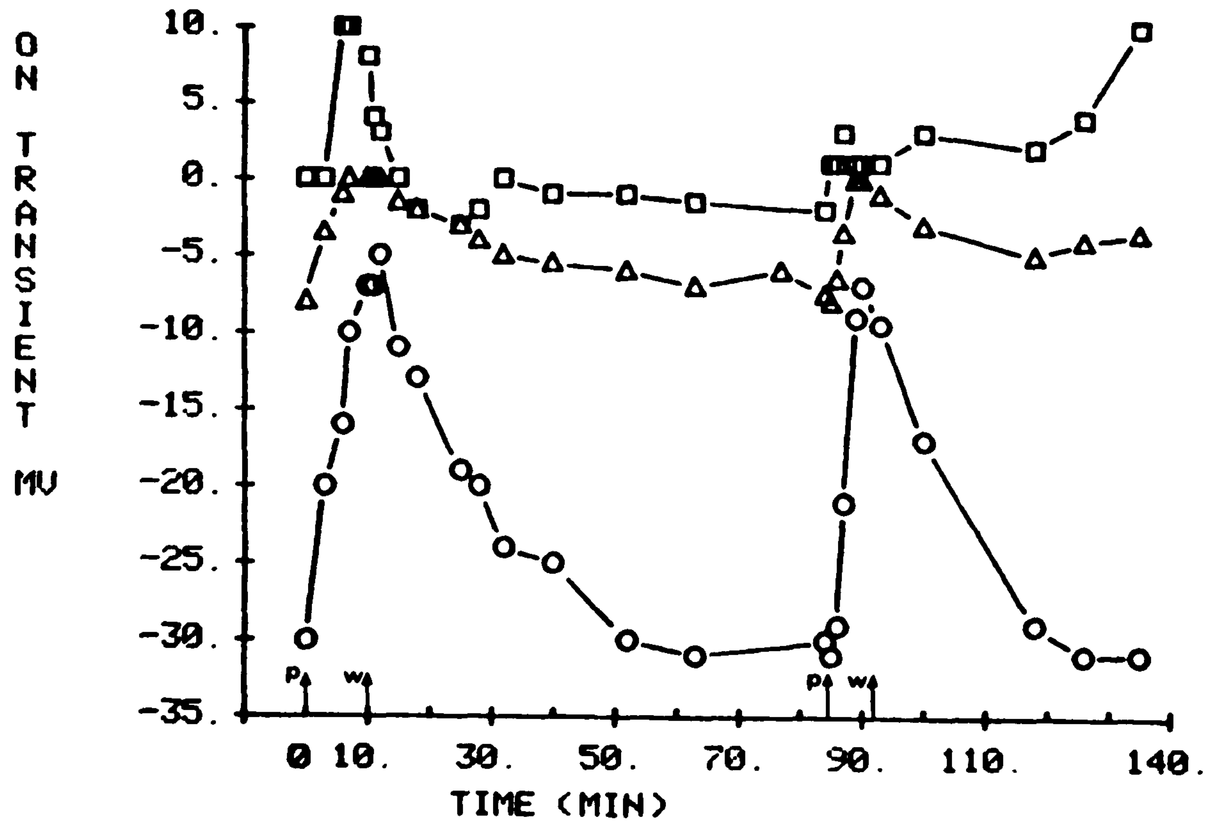
Effect of 2 consecutive applications of 10 mM carbachol on the ON-transient, sustained response and dark potential of the L-neuron (see Graphs XXXVII, XXXVIII, and XXXIX). The response of the L-neuron is plotted as a function of time. This data was obtained from the experiments shown in figures 64 and 65. Carbachol reduced the amplitude of the ON-transient and sustained response and depolarized the L-neuron during 2 consecutive applications of carbachol. The amplitude of the ON-transient and sustained response to light were measured from the pre-flash dark potential. The change in the dark potential was measured with respect to the dark potential at time 0, before carbachol perfusion was initiated.

Stimulus intensity: Log I = -3.

Ordinate: Amplitude of L-neuron response during carbachol perfusion. The dark potential before the application of carbachol corresponds to 0 millivolts on the ordinate scale.

Abscissa: Time course of carbachol perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.

(See note on pp. 378.)



Graph XL

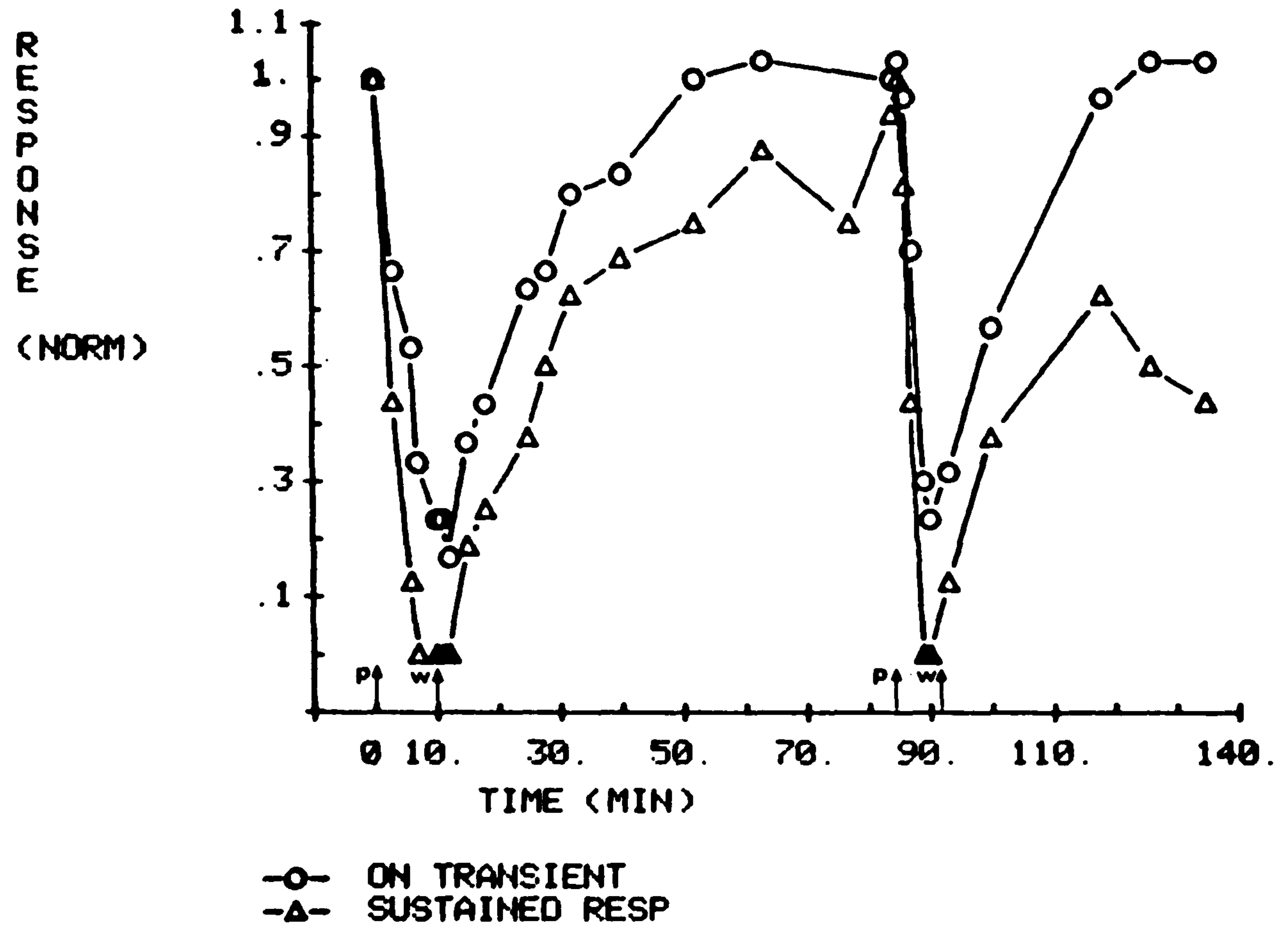
Graph XLI

Effect of 2 consecutive applications of 10 mM carbachol on the ON-transient and sustained response of L-neuron. This is the same data shown in the preceding graph (Graph XL) except the responses have been normalized by dividing the amplitude of each response (mv) at various intervals during the time course of the experiment by their respective values at time 0, before carbachol perfusion was initiated. The normalized responses are plotted as a function of time. This data was obtained from the experiment shown in figures 64 and 65. Carbachol reduced the amplitude of the ON-transient and sustained response of the L-neuron during 2 consecutive perfusions and washes. The relative decrease in the amplitude of the response was greatest for the sustained component.

Stimulus intensity: Log I = -3.

Ordinate: Normalized ON-transient and sustained components of L-neuron light responses. The responses were measured with respect to the dark potential prior to light-ON.

Abscissa: Time course of carbachol perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XLI

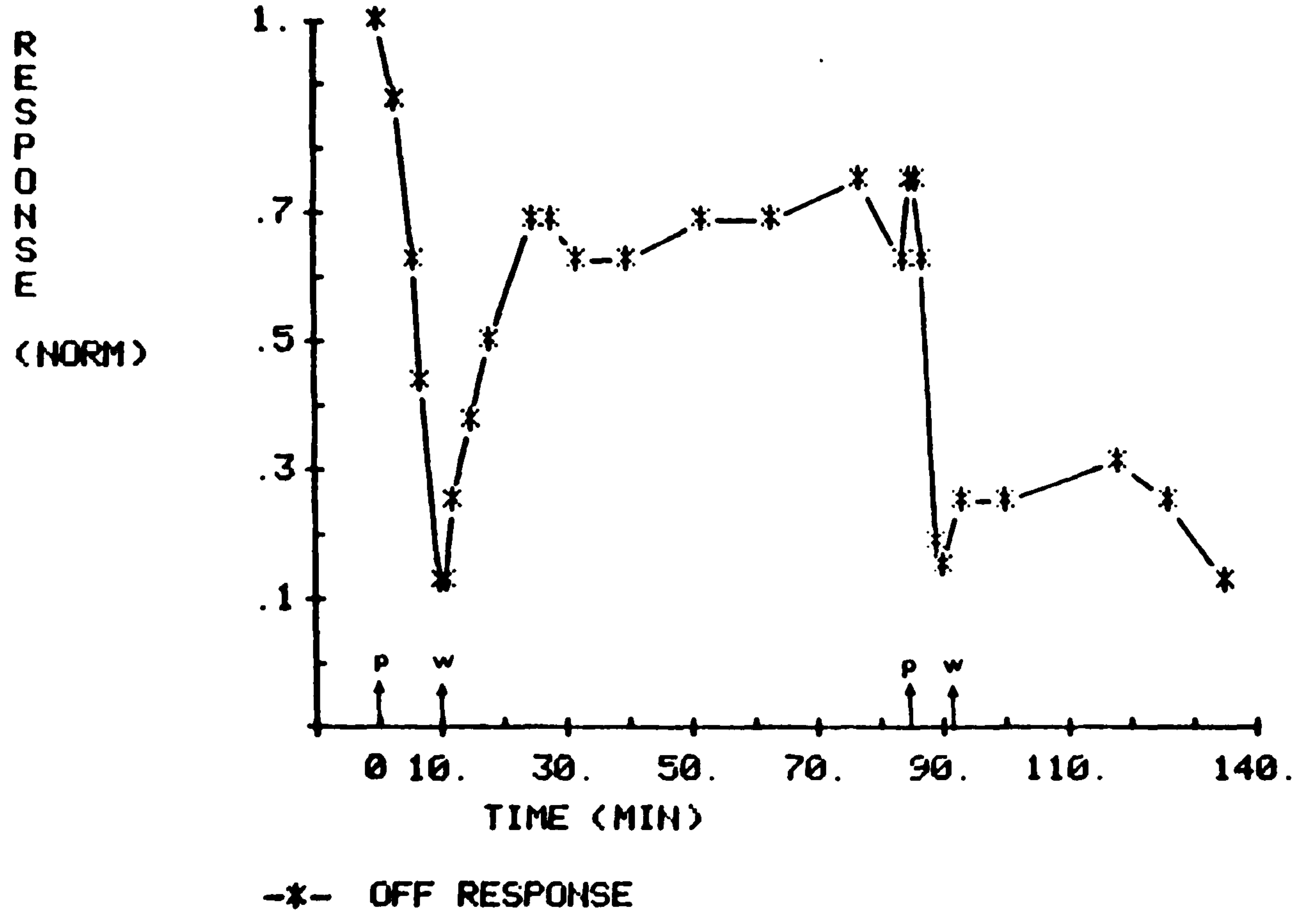
Graph XLII

Effect of 2 consecutive applications of 10 mM carbachol on the depolarizing OFF-response of the L-neuron. The OFF-response was normalized by dividing the amplitude of each response (measured in millivolts with respect to the pre-flash dark potential) at various intervals during the time course of the experiment by the value at time 0, before carbachol perfusion was initiated. The normalized OFF response is plotted as a function of time. This data was obtained from the experiment shown in figures 64 and 65. Carbachol reduced the amplitude of the OFF response during 2 consecutive perfusions. Note that dark potential was at a more depolarized level at this time (see Graphs XXXIX and XL).

Stimulus intensity: Log I = -3.

Ordinate: Normalized OFF response (measured from the pre-flash dark potential).

Abscissa: Time course of carbachol perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XLII

Graph XLIII

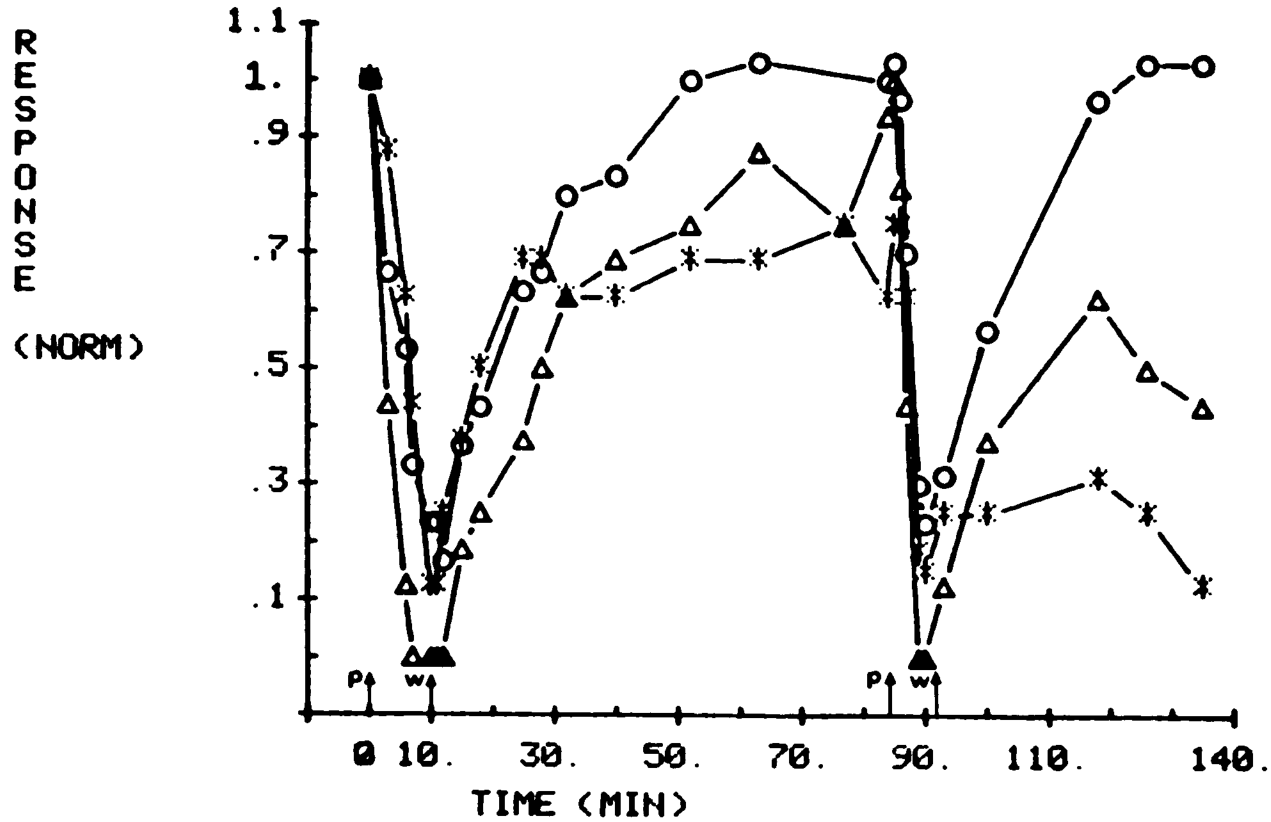
Effect of 2 consecutive applications of 10 mM carbachol on normalized ON-transient, sustained, and OFF-response of L-neuron. The responses were normalized by dividing the amplitude (in millivolts) at various intervals during the time course of the experiment by the respective response amplitudes at time 0, before carbachol perfusion was initiated. The normalized responses are plotted as a function of time. This data was obtained from the experiment shown in figures 64 and 65. Carbachol reduced the amplitude of all 3 components of the L-neuron response. The responses were measured as described in Graphs XXXVII , XXXVIII, and XLII. The sustained response showed the largest relative reduction in amplitude during carbachol perfusion, but the ON-transient response showed the most complete recovery during the wash period.

Stimulus intensity: Log I = -3.

Ordinate: Normalized L-neuron response measured with respect to the pre-flash dark potential.

Abscissa: Time course of carbachol perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.

CARBACHOL (X 2) TO L-NEURON #2



-o- ON TRANSIENT
-Δ- SUSTAINED RESP
-*- OFF RESPONSE

Graph XLIII

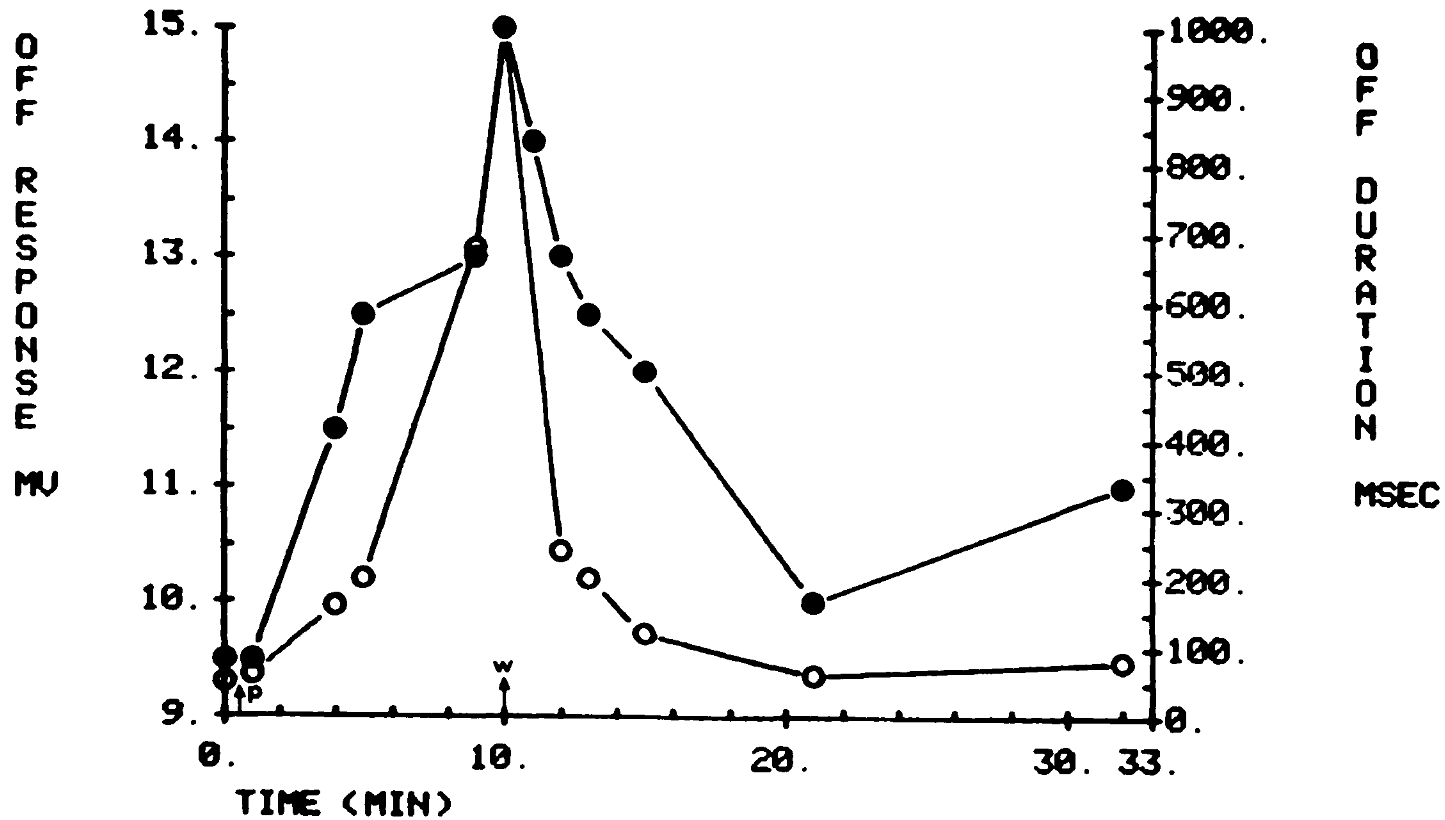
Graph XLIV

Effect of 1.6 mM picrotoxin on the amplitude and duration of the receptor OFF response from an intact nerve preparation. The amplitude (Left-Y-axis) and duration (Right-Y-axis) are plotted as a function of time. This data was obtained from the experiment shown in figures 66, 67 and 68. The amplitude of the OFF-transient was measured from the sustained response to light (see D in figure 83). During the application of picrotoxin the receptor OFF-response increased in amplitude and duration.

Stimulus intensity: Log I = -2.

Ordinate: (Left-Y-axis) Amplitude of receptor OFF-transient measured from sustained portion of the light response ("mv from sust"). (Right-Y-axis) Duration of receptor OFF-response (msec).

Abscissa: Time course of picrotoxin perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



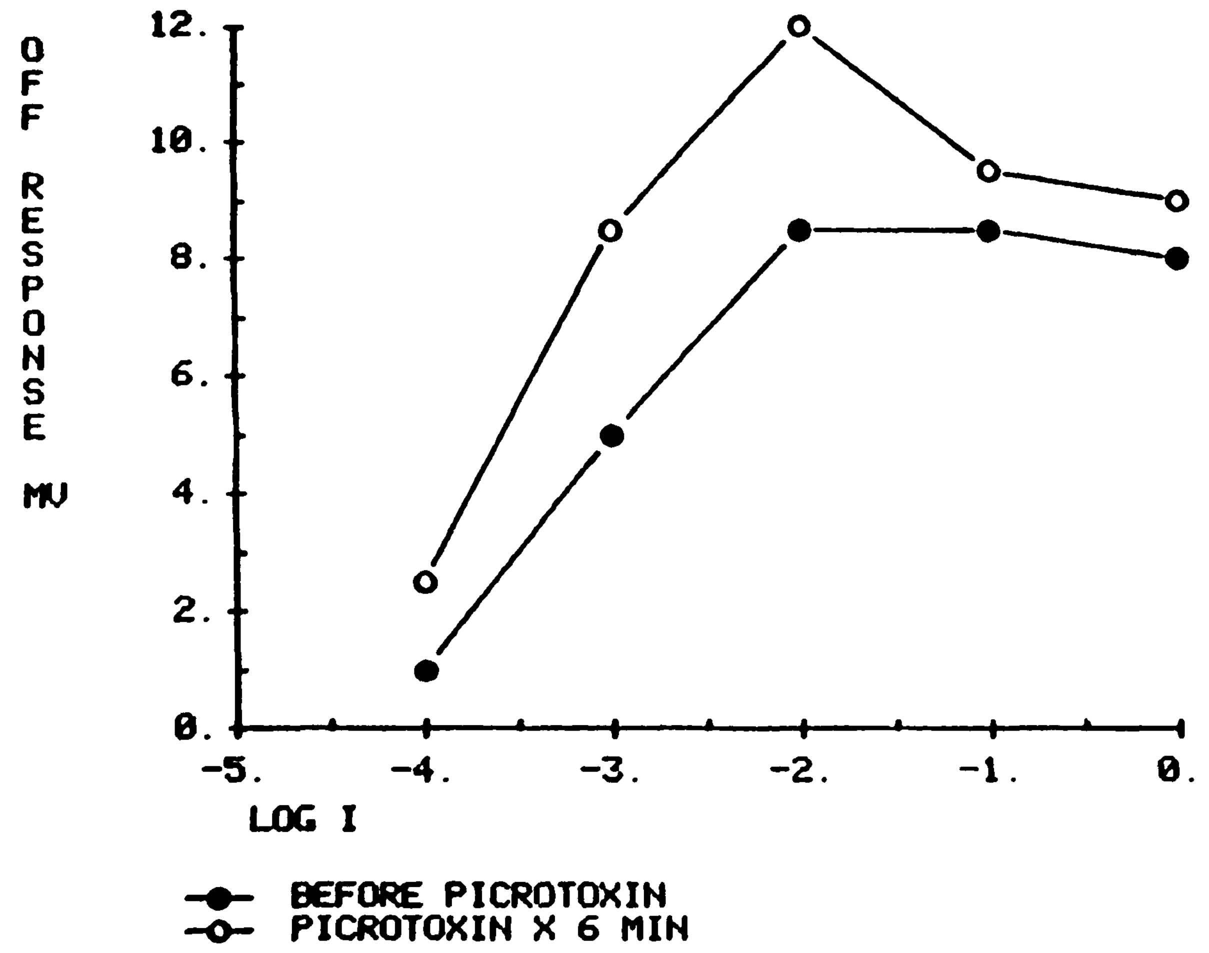
Graph XLIV

Graph XLV

Effect of 1.6 mM picrotoxin on receptor OFF-response from intact nerve preparation. The amplitude of the OFF-response (millivolts from sustained light response) is plotted as a function of stimulus intensity (Log I). This data was obtained from the experiment shown in figures 66-70. The increase in the amplitude of the OFF response during picrotoxin perfusion was greatest for moderate stimulus intensities.

Ordinate: Amplitude of receptor OFF-transient (millivolts) measured from sustained portion of light response (see D in figure 83 and Graph I).

Abscissa: Intensity of illumination (Log I).



Graph XLV

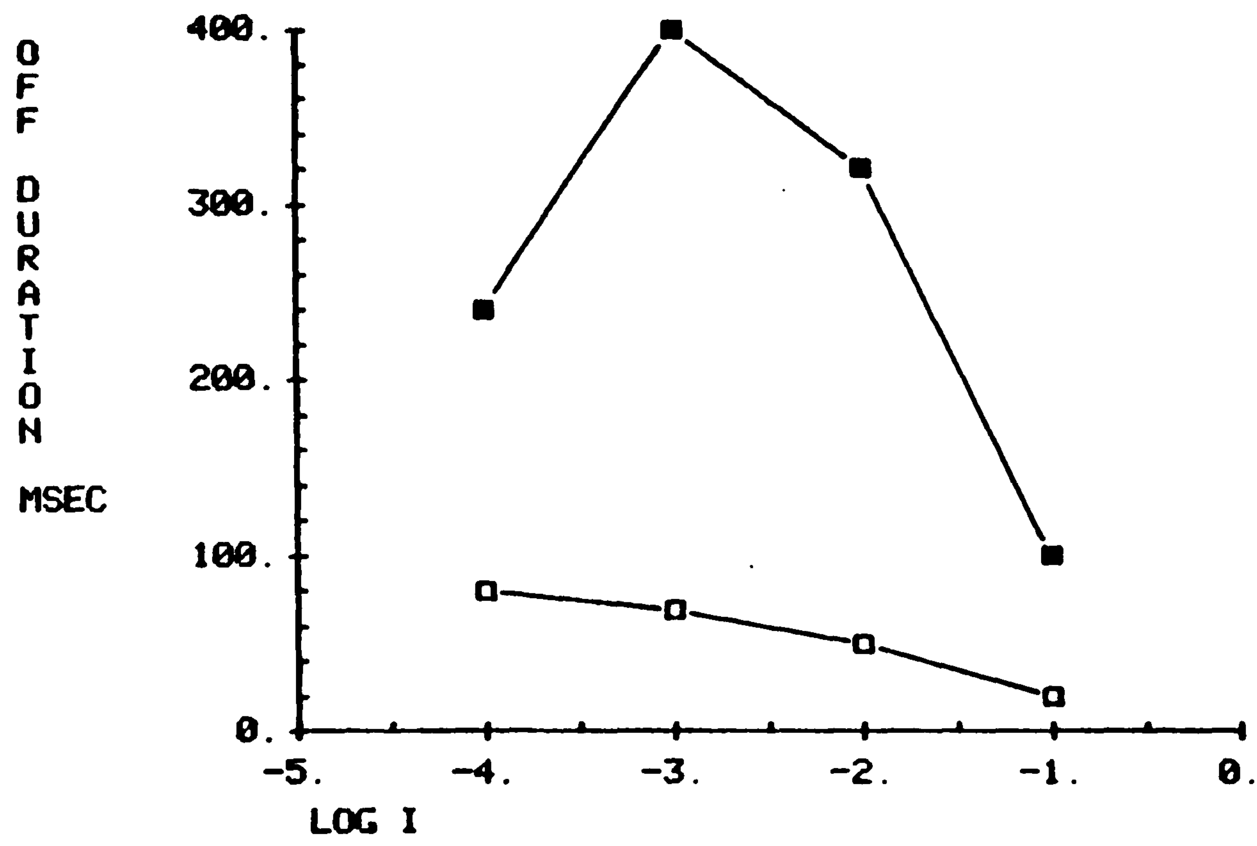
Graph XLVI

Effect of 1.6 mM picrotoxin on duration (milliseconds) of receptor OFF-response from intact nerve preparation. The duration of the hyperpolarizing OFF-transient is plotted as a function of stimulus intensity (Log I). This data was obtained from the experiment shown in figures 66-70. The increase in the duration of the OFF response during picrotoxin perfusion was greatest for moderate light intensities.

Ordinate: OFF-response duration (milliseconds).

Abscissa: Intensity of illumination (Log I).

PICROTOXIN TO RECEPTOR



—□— OFF DUR BEFORE PICROTOXIN
—■— OFF DUR (PICROTOXIN X 6 MIN)

Graph XLVI

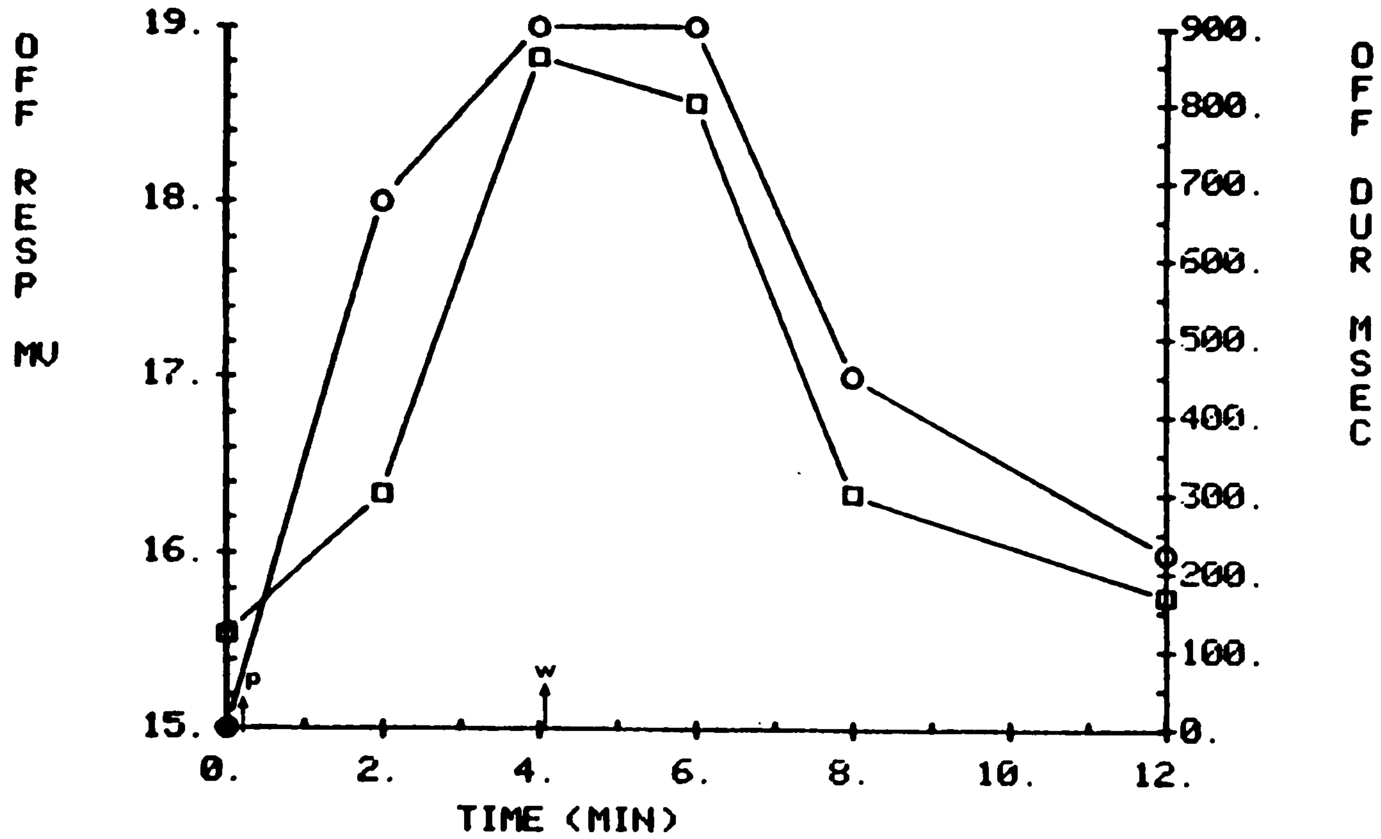
Graph XLVII

Effect of 1 mM picrotoxin on amplitude and duration of receptor OFF response from cut nerve preparation. The amplitude (Left-Y-axis) and duration (Right-Y-axis) are plotted as a function of time. This data was obtained from the experiment shown in figure 71. The receptor OFF-response reversibly increased in amplitude and duration during the application of picrotoxin.

Stimulus intensity: Log I = -2.

Ordinate: (Left-Y-axis) Amplitude of receptor OFF-transient (millivolts) measured from sustained portion of the light response (see D in figure 83 and Graph I). (Right-Y-axis) OFF-response duration (milliseconds).

Abscissa: Time course of picrotoxin perfusion experiment (minutes). Perfusion and wash times are indicated by arrows.



Graph XLVII