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*City University of New York* PH.D. 1983

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# ELECTROPHYSIOLOGY AND PHARMACOLOGY OF DRAGONFLY OCELLAR NERVE IMPULSE ACTIVITY

by

Jeffrey D. Rind

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.

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August 20, 1983
date

Richard L. Chappell
Chairman of Examining Committee
Dr. Richard L. Chappell, Hunter College

September 15, 1983
date

Alex E. Bard, Deputy
Executive Officer for James Engel, E.O.

William D. Cohen
Dr. William D. Cohen, Hunter College

Joseph Goldfarb
Dr. Joseph Goldfarb, Mt. Sinai Med. Sch.

William Ross
Dr. William N. Ross, N.Y. Medical College

R. Bruce Szamier
Dr. R. Bruce Szamier, Marine Biological Lab

Supervisory Committee

The City University of New York

## Abstract

ELECTROPHYSIOLOGY AND PHARMACOLOGY OF DRAGONFLY OCELLAR NERVE IMPULSE ACTIVITY

by

Jeffrey D. Rind

Advisor: Professor Richard L. Chappell

A preparation has evolved which utilizes extracellular recordings of the light inhibited impulse activity from the cornea of the dragonfly ocellus to study the pharmacology of the ocellus. Computer software was developed which collects and analyzes spike frequency data. Ablation studies suggest that the impulse activity recorded in this fashion is originating from the lateral ocellar nerves and not the median ocellar nerve. TTX blocks all impulse activity. Latencies between action potentials suggest that there is only one spiking cell so recorded per lateral ocellar nerve that fires light inhibited spikes. Impulse activity appears to be more sensitive to green light (484 nm) than it is to UV light (360 nm) and unlike the ocellar photoreceptors or L-neurons it does not exhibit a reverse Purkinje shift at higher light intensities.

Intracellular recordings from the lateral ocellar nerve reveal non-spiking cells that have L-neuron like light responses (resting potentials = -30 to -55 mv). The spiking

neuron (resting potential= -55 mv) fires light inhibited spikes analagous to those recorded extracellularly. The amplitude of the impulses was 30 mv. Recordings of extracellular impulse activity made simultaneously with an intracellular recording from an L-neuron suggest that the slow potential responses of the L-neuron may be normal for that cell and that it is not a damaged spiking neuron.

Agents that have been reported to effect the L-neuron also effect the corneally recorded impulse activity. 50 uM curare lowered the response threshold of the light response and decreased the interflash spike frequency. 50 uM curare also changed the slope of the intensity-freqency relationship. It has been suggested that these effects are mediated by curare's effect on the presumptive lateral inhibitory interactions between photoreceptors. Increasing doses of curare greater than 100 uM block the light mediated inhibition of impulses and cause an increase in the interflash spike frequency and spontaneous dark adapted impulse rate.

GABA inhibited spontaneous impulse activity in the dark and caused a light dependent increase in spike frequency which is shifted towards light "off" at high intensities. 12 mM cobalt, an inhibitor of synaptic transmission blocked all impulse activity.
A model was suggested to explain the data which proposes that spiking activity occurs between two thresholds.

To my mother, father, and sister.

## Acknowledgements

I would not at this moment be writing the acknowledgement section to my thesis if it were not for my advisor Professor Richard L. Chappell. On the day that I met him he took me into his lab, no questions asked, and gave me an opportunity to find myself. My success is his success and I will always be grateful.

I would like to single out Professor J. Goldfarb who next to my advisor had the greatest hand in my scientific training.

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continued.....

# INTRODUCTION

In 1926, Adrian and Zotterman recorded extracellularly from a frog's sensory nerve and demonstrated that information appeared to be encoded in the frequency of the nerve impulse. After intracellular recordings became possible, Katz (1950) provided evidence that the frequency of impulses in a first order sensory neuron is proportional to the magnitude of the intracellular potential change recorded from the receptor. This led to a general understanding among sensory physiologists that the change in the impulse firing rate of a sensory neuron is modulated by the receptor potential whose amplitude is proportional to the magnitude of the stimulus. However, many sensory systems contain a number of cells, in addition to the primary receptors, which act to modulate the frequency of impulses seen in their principle neurons (Werblin, 1979; Calvin and Graubard, 1979). A well known example of such systems is provided in the cellular organization of the vertebrate retina (Dowling, 1979).

Vertebrate photoreceptors respond to light with intensity-dependent, graded slow potentials (Bortoff, 1964; Tomita, 1968; Werblin and Dowling, 1969;). These potentials are integrated by a network of neurons which pre-

serve and encode visual information. As in many other sensory systems this information is conducted along the optic nerve towards the brain in the form of regenerative spikes. However, in this case the frequency is modulated not only by the intensity, but also by factors concerned with image quality, orientation, motion, and wavelength (Werblin, 1979; Miller, 1979; Dowling, 1979). This means that the variations of impulse frequency which can be seen in each individual axon, incorporates many different types of information.

Anatomical and electrophysiological studies have demonstrated that within the vertebrate retina there are two layers of synaptic plexes in which at least five general categories of cells interact (Cajal, 1894; Dowling and Boycott, 1966; Dowling and Werblin, 1969; Werblin and Dowling, 1969). Recent studies have demonstrated that there are many sub-types within each category (Kolb and Famiglietti, 1974; Miller, 1979; Werblin, 1979; Ashmore and Copenhagen,1980). This structural and functional complexity makes it difficult to discern the physiological means by which the encoding of the visual information takes place.

This thesis is concerned with the complexities of encoding sensory stimuli in a simple photosensitive system.

The investigation of this system was carried out by pharmacologically modifying the impulse firing rate of an afferent neuron under different physiological conditions. It should be noted that in order to study the modulation of action potential frequency by an integrated network of neurons, a model system is needed which is both simple enough to manipulate experimentally but sophisticated enough so that parallels can be drawn between it and more complicated cellular networks. Invertebrates offer convenient systems in which to study the nature of these processes (Shaw, 1979). For example, the ocelli of the barnacle (Gwilliam, 1965; Stuart and Oertal, 1978), the locust (Patterson and Goodman, 1975; Wilson, 1978), and the dragonfly (Ruck, 1961; Chappell and Dowling, 1972) contain a few types of neurons which are large in comparison to most vertebrate cells and are accessible to both stable intracellular recording and extracellular analysis.

Dragonfly ocelli, besides offering a convenient means in which to record ocellar nerve inpulses (Ruck, 1961; Chappell and Dowling, 1972), appear to contain only two basic types of neurons, the receptors and second order cells (Ruck and Edwards, 1964; Dowling and Chappell, 1972), which may be communicating with each other via lateral and reciprocal synapses (Dowling and Chappell, 1972; Stone and

Chappell, 1981). These cells appear to communicate without the use of impulse activity (Pearson, 1979). This is reminiscent of some aspects of the synaptic organization of the plexiform layers of the vertebrate retina, but on a smaller scale (Werblin and Dowling, 1969; Miller, 1979; Gerschenfeld and Picolino, 1979).

Most of the preceding experiments concerned with the electrophysiology and pharmacology of the dragonfly's ocellar system were carried out in the median ocellus. Unlike the nerves of the lateral ocelli, the median ocellar nerve does not appear to contain neurites carrying the large action potentials that can be routinely recorded with an extracellular electrode. However, intracellular recordings, from the second order cell dendrites (L-neurons) of the lateral ocellus are analogous to those seen in the median ocellus. Compare figure 12 of (Chappell and Dowling, 1972) to figure 11B, of this thesis. Also, electroretinograms (ERG) recorded from any of the ocelli are indistinguishable from each other (Ruck, 1958, 1961). This is consistent with the histological observations that the lateral and median ocelli contain a similar anatomical organization (Ruck and Edwards, 1964).

Recent pharmacological studies carried out in the med-

ian ocellus have provided sufficient data for the formulation of a model which can account for the effects of neuronal integration on the slow, light evoked, potentials (Klingman, 1976; Klingman and Chappell, 1978; Stone and Chappell, 1981; Stone, 1982). However, the difficulties of maintaining an intracellular electrode within a cell limits the time in which one can perfuse and wash a preparation before that cell is lost or irreparably damaged. Extracellular recording techniques can provide further information because they offer the advantage of being able to record impulse activity for as long as the preparation remains viable.

Recordings of ocellar nerve action potentials from the ocellar cornea are easy to obtain, are non-invasive, and presumably reflect the integrative activity of all of the neurons which input into the ocellar plexus (Ruck, 1961; Chappell and Dowling, 1972; Kondo, 1978). Also, it was found that one can perfuse and record from the preparation for a number of hours before any deterioration becomes apparent.

The use of extracellular ocellar nerve recordings to study the properties of the ocellus has a precedent based on the classical studies of visual physiology in the Limulus compound eye (Ratliff, 1974). Extracellular re-

cordings from the Limulus optic nerve were used to study the approximately linear relationship between the intensity of illumination and the receptor response (Hartline and Graham, 1932), the reciprocity of intensity and duration (Hartline, 1934), spectral sensitivity (Graham and Hartline, 1935), light and dark adaptation (Hartline and McDonald, 1947), and the lateral interactions between neighboring neurons (Hartline, et. al., 1952).

Evidence has been published which strongly suggests that the light inhibited action potentials of the lateral ocellar nerve are afferent in nature (Ruck, 1961; Kondo, 1978), and their inhibition may reflect the dendritic summation of synaptic potentials from a number of different presynaptic units in the dragonfly ocellar system (Ruck and Edwards, 1964; Dowling and Chappell, 1972; Kondo, 1978)(see Background Information, Impulse Activity section).

Since the ocellar nerve impulse activity appears to represent the output of a relatively simple integrating network, these studies should contribute to the understanding of how such networks of synaptically connected cells operate. This information may also be helpful in understanding the interactions of larger neuronal networks that have already been partially studied, but whose pro-

cessing pathways have not as yet been completely worked out.

# BACKGROUND INFORMATION

## Anatomy

Many insects contain photosensitive organs, distinct from their compound eyes, called ocelli (Dethier, 1942; Parry, 1947; Cornwall, 1955; Hoyle, 1955; Goodman, 1970; Pappas and Eaton, 1977). In the dragonfly, three antero-dorsal ocelli are present on the head of the animal (figure 1). Ocellar dimensions reported from animals in the genera Anax and Aeschna show the median ocellus to be elliptical in shape, measuring approximately 0.9 mm along the horizontal major axis and 0.55 mm along the minor axis. The lateral ocelli appear to be more circular with a diameter of 0.2 mm (Chappell, et. al.,1978).

Histological sections show that the dragonfly ocellus is organized in discrete layers. The most distal is the layer of elongated photoreceptors and tapetal sheath cells. Both of these cells are located just under the cuticular corneal lens (figure 2)(Ruck and Edwards, 1964). The photoreceptors individually measure 300 to 400 microns in length, 15 microns in diameter, and are

usually grouped in threes to form an organized structure called an ommatidium (Ruck, 1962; Ruck and Edwards, 1964; Chappell and Dowling, 1972). The distal photosensitive part of the ommatidium and of each photoreceptor cell which belongs to it are called the rhabdome and rhabdomere respectively. The nuclei of the photoreceptors lie just proximal to the rhabdomeric region, and give the appearance of being pseudostratified.

Each ommatidium is ensheathed throughout its entire length by the cytoplasm of tapetal cells. The pigment granules seen in these tapetal cells give the ocellus its white appearance when viewed head on, even though most of the granules are found in the region between adjacent photoreceptor cell bodies (Ruck and Edwards, 1964).

Proximal to the layer of nuclei, one can see the processes of tapetal sheath cells interdigitating with the processes of a layer of pigment cells which contain darker pigment granules. Photoreceptor cells send axons through this layer of pigmented epithelium (Patterson and Chappell,1980), into a region where they make synapses onto ocellar nerve dendrites and other receptor cells ( Ruck and Edwards, 1964; Dowling and Chappell, 1972; Patterson and Chappell, 1980).

## Synaptic organization

Two types of synapses have been recognized in the synaptic plexus (Dowling and Chappell, 1972). The most common type, called a "button synapse", contains a densely staining button-like organelle in the presynaptic cytoplasm. This organelle is reminiscent of the synaptic ribbons seen in the bipolar cell and receptor cell terminals of the vertebrate retina (Dowling and Chappell, 1972). There are two postsynaptic elements for each button synapse. The postsynaptic elements usually consist of one receptor terminal and one ocellar nerve dendrite, whether the presynaptic process comes from an ocellar nerve fiber or a photoreceptor cell (Dowling and Chappell, 1972). There are also reciprocal synaptic contacts between ocellar nerve dendrites and receptor terminals (Dowling and Chappell, 1972). These anatomical structures support the idea that lateral interactions exist between ocellar nerve dendrites and between neighboring retinula cells and is consistent with the feedback model of synaptic interaction which was first proposed by Klingman and Chappell (1976) and later modified by Stone and Chappell (1981). The model is described in the pharmacology section of this introduction.

The second type of synapse does not contain "buttons" or "ribbons", and is referred to as a conventional synapse (Dowling and Chappell, 1972). Conventional synapses contain only one postsynaptic element. They are found most commonly in receptor cell terminals and are only occasionally seen in the ocellar plexus (Dowling and Chappell, 1972).

In all three ocelli, large numbers of retinula cells converge onto small numbers of ocellar nerve fibers. For example, the retina of the lateral ocellus contains approximately 675 retinula cells (Cajal, 1918) while the lateral ocellar nerve appears to have only 20 to 30 fibers (Ruck and Edwards, 1964; Chappell et. al., 1978). The synapses made by many retinula cell axons onto the small number of nerve fibers has been suggested as a possible explanation for the finding that the L-neuron (ocellar nerve dendrites) has an apparent sensitivity to light, which is 2 log units greater than that of the photoreceptor (Chappell and Dowling, 1972).

## Ocellar nerve projections

There appear to be 4 large fibers in the lateral ocellar nerve with diameters which range in size from 15 to

30 microns. These fibers are referred to as L-neurons because of their relatively large diameters and because of their location in the lateral ocellar nerves (Wilson, 1978). The smaller fibers have diameters measuring as low as 5 microns (Ruck and Edwards, 1964;Chappell, et. al., 1978). In Aeschna, the lateral ocellar nerve is reported to be 1.25 mm in length, when measured from the proximal portion of the ocellar cup to the brain, and 60 microns in diameter (Chappell, et. al., 1978). It maintains a constant diameter throughout most of its length. The shorter median ocellar nerve measures 0.55 mm in length, and although it is bilobed near the ocellar plexus, it forms a single nerve as it approaches the brain (Chappell, et. al., 1978; Zenkin and Pigarev, 1971). At the point at which it forms a single nerve it attains a maximum diameter of about 200 microns decreasing to approximately 150 microns where it penetrates the brain (Chappell, et. al., 1978.

The cell bodies of the neurites found in all three ocellar nerves, are located in the brain as demonstrated by preparations stained with cobalt and procion dyes (Chappell, et. al., 1978; Patterson and Chappell, 1980).

The lateral ocellar nerves enter the brain adjacent to the median nerve at which point all three nerves merge

into a single tract. The bundle of fibers passes through the pars-intercerebralis and descend towards the protocerebral bridge. It has been shown that of the four large fibers seen in each lateral ocellar nerve, two project and arborize in the ipsilateral posterior neuropile of the protocerebrum, and two arborize in the contralateral posterior neuropile (Chappell, et. al., 1978). Presumably the branching fibers make synaptic contact with the neurons of the brain.

Nine unique median ocellar nerve L-neuron projections have been identified (Patterson and Chappell, 1980). One of the median ocellar nerve fibers projects to both sides of the brain, and sends processes to both lateral ocellar nerves (Patterson and Chappell, 1980; Mobbs, et. al., 1981). The axon terminals of some of the median ocellar neurons appear to branch in the same region at which the lateral ocellar nerve fibers branch. This observation is consistent with the idea that the ocelli communicate with each other (Kondo, 1978)(see Background Information: Impulse Activity).

## Electroretinogram

Ruck (1961) distinguished the contributions of four components to the waveform of the ocellar Electroretinogram (ERG) in cockroach and dragonfly. Component 1, a tonic negative potential present during illumination and component 2, a single transient "on" spike, are both generated by the retinula cells. Component 4, the spontaneous impulse activity, and component 3, the notch in the initial depolarization of the ERG, are generated by the ocellar nerve and its dendrites respectively. This implies that component 3 is produced by the sustained hyperpolarization of the ocellar nerve dendrites, and that component 1 results from the light evoked depolarization of the photoreceptors (see the next section of this introduction). Since the polarity of the impulse activity (component 4) is always positive, whether it is recorded from the cornea (ERG) or the distal ocellar nerve, component 4 appears to be originating at some point proximal to the ocellar nerve dendrites.

An "off" response can be seen in both cockroach and dragonfly ERGs (Ruck, 1961). It appears as a single spike or burst of spikes and/or a positive going oscillation which does not always occur at the highest light

intensities. Ruck felt that this effect was due to synchronous firing of spikes in the ocellar nerve fibers (Ruck, 1961), but other studies suggest that it reflects the composite "off" response of the ocellar neurons (Chappell and Dowling, 1972; Klingman and Chappell, 1978).

## Intracellular recording

Intracellular recordings from ocellar neurons have demonstrated that the photoreceptors respond to light with a graded, sustained depolarization, and a single regenerative spike at light "on". During high intensity illumination, the sustained depolarization is preceded by a transient depolarization which occurrs after the initial "on" spike (Chappell and Dowling, 1972). The "on" spike has a duration of 3 milliseconds and it's presence can be eliminated with tetrodotoxin (TTX). This suggests that the spike is caused by an increase in sodium conductance.

Chappell and Dowling (1972) have brought attention to the "oscillatory 'off' wave", which they called an unusual feature of the dragonfly ocellar photoreceptor response. This type of "off" response has not been noted in photoreceptors of other invertebrates (Dowling

and Chappell, 1972). At low intensities, the "off" response is often the most prominent aspect of the response (Chappell and Dowling, 1972).

The L-neurons respond to light with a sharp transient hyperpolarization followed by a relatively small sustained hyperpolarization beyond the dark membrane potential. This small graded sustained hyperpolarization lasts for the duration of the light stimulus. Since TTX does not appear to change the light response of the L-neuron, while it does inhibit the receptor "on" spike, it seems reasonable to conclude that synaptic transmission is mediated by slow potentials.

At light "off", there is a sharp overshoot followed by a less severe undershoot of the dark potential (Klingman,1976). It would appear that this "off" overshoot could be related to the corresponding "off" response seen in the photoreceptors (Chappell and Dowling, 1972).

The ratio of the initial transient response amplitude to that of the sustained response is much greater in the L-neuron than in the photoreceptor, indicating the phasic nature of information processing at this synapse. Also postsynaptic activity appears to be more sensitive to light flashes by up to 2 log units (Chappell and Dowling,

1972). It has been suggested that lateral and feedback synaptic connections, which have been demonstrated anatomically, integrate the information received by the L-neurons and result in its relatively transient responses and increased sensitivity to light (Chappell and Dowling, 1972).

In a spectral sensitivity study on median ocellar photoreceptors, half of the cells studied were more sensitive to green light than they were to ultraviolet light at threshold. This investigation revealed that as the intensity of illumination is increased, the intensity response curves, at these two wavelengths, cross, so that a greater intensity (quantum flux) of green light is needed to evoke a response equal in magnitude to a response evoked by a less intense flash of UV light (Chappell and DeVoe, 1975). This crossing of the intensity response relationship is referred to as a "reverse Purkinje shift" (Chappell and DeVoe, 1975). A similar study on the median ocellar L-neuron has demonstrated a reverse Purkinje shift in this cell also (Mobbs, et. al., 1981). One of the objectives of my investigation was to determine if such a shift occurred for the impulse activity.

## Impulse activity

A number of investigators have studied the dragonfly ocellar impulse activity (Ruck, 1958a, 1961; Rosser, 1974; Kondo, 1978). In uncut nerves it was found that there are at least two populations of spikes. The larger spikes are inhibited by ocellar illumination whereas the frequencies of the smaller spikes is enhanced by it (Kondo, 1978). In addition, the frequency of firing increased for both types of spikes during the wingbeat of the animal. Recordings from the cut end of the ocellar nerve after it has been sectioned near the brain have revealed that only the larger light inhibited spikes are present (Kondo, 1978, Ruck, 1961), while only the smaller light facilitated spikes can be recorded from the nerve stump protruding from the brain (Kondo, 1978; Rosser, 1974). This is consistent with the notion that the light inhibited action potentials represent afferent activity, and the light facilitated spikes represent efferent activity (Ruck, 1961; Kondo, 1978; Chappell and Dowling, 1972).

Although it is presently unclear as to what role the ocellus plays in the animals' behavior and physiology

(Goodman, 1970; Wilson, 1978), a number of investigators have suggested that it is a sense organ used to maintain the orientation of the animal in flight (Wilson, 1978; Stang and Howard, 1979).

For example, in cut nerve preparations, efferent activity is enhanced by illumination of the compound eyes, by wing movement, and by electrical stimulation of the ipsilateral and contralateral wing sensory nerves (Kondo, 1978). When wing sensory nerve activity is eliminated by sectioning the subesophogeal connectives, the wingbeat has no effect on the efferent discharge. Also, Unequal illumination of the ocelli results in head rolls consistent with the animals need to maintain an orientation in parallel with the horizon (Stange and Howard, 1979).

Another investigator has suggested that in Limulus, the median ocellus may be acting to adjust the sensitivity of the compound eye under certain conditions (Barlow, et. al., 1977). In the dragonfly, the ocelli and compound eyes may work together to adjust the animal's overall sensitivity to light. This is suggested by the following experiments. When recording from the proximal cut end of one of the lateral ocellar nerves, illumination of the other two ocelli and the compound eye increases the presumed efferent activity, with notable "on" and "off"

bursts at high stimulus intensities. When the other two ocelli are removed, illumination of the compound eye still enhances this efferent activity. However, there is a reduction in the magnitude of the "off" response. This suggests that at least one of the presumed efferent fibers in one lateral ocellar nerve receives excitatory input, within the brain, from afferent fibers in the other ocellar nerves (Kondo, 1978).

Impulse activity can be recorded from the corneas of any of the three ocelli. However, intracellular recordings from the median ocellus rarely demonstrate any spiking activity. This is particularly puzzling in light of the previously described experiments. Therefore, one of the objectives of this study was to determine the source of the impulses monitored in corneal recordings. This information might help in discerning the function of the ocellus.

## Pharmacology

Tetrodotoxin sensitive spikes have been identified in a number of invertebrate visual systems (Stuart and Oertel, 1978; Wilson, 1978). Tetrodotoxin (TTX) sensitivity is a distinctive characteristic of impulse activity that is mediated by Na currents (Armstrong and Benzanilla, 1974). It has been shown that tetrodotoxin reduces the amplitude of the receptor "on" spike without affecting synaptic transmission (Chappell and Dowling, 1972). Ruck (1961) had suggested that the ocellar "off" response observed in the ERG might be a reflection of synchronous firing of ocellar nerve impulses (see Background Information: Electroretinogram). Since the "off" transient of L-neurons survive TTX, it could not be a response to impulse activity if impulses are blocked by TTX. Therefore another objective of this study was to determine what effects, if any, tetrodotoxin has on ocellar nerve impulse activity.

Curare, a competitive inhibitor of nicotinic cholinergic receptors, was shown to reversibly block the light induced hyperpolarizing response of the L-neuron without affecting the light response of the photoreceptor (Chappell and Dowling, 1972; Klingman and Chappell,1978; Stone,1982).

Curare also prolonged the the photoreceptor's "off" response (Stone and Chappell, 1982). When Atropine, a muscarinic cholinergic antagonist, was applied to the preparation no comparable effects were observed (Klingman,1976).

These experiments suggest that the photoreceptor terminal transmitter is acetylcholine, that it is released during the time of illumination, and that the postsynaptic receptors are nicotinic in character. It has been reported that significant amounts of acetylcholine and choline acetyltransferase activity can be seen in assays of ocellar tissue (Chappell and Kuhar, 1976). This observation further butresses the argument that there is cholinergic activity in this organ. Since the L-neurons respond to light with a hyperpolarization and an increase in conductance, it appears that the photoreceptor transmitter has an inhibitory effect upon them. It should be noted that this idea is in agreement with Ruck's analysis of the ERG waveform (Ruck, 1961)(see Background Information: Electroretinogram).

Carbachol, a nicotinic cholinergic agonist that is resistant to acetylcholinesterase hydolysis (Koelle, 1975a), was shown to reduce the amplitude of both the "on" transient and sustained portion of the light response and cause a passing increase in the size of the "off" response

of the L-neurons. Carbachol was also shown to depolarize the membrane potential of this cell in the dark (Stone, 1982). These effects could be reversed by washing in Ringer. Since Carbachol mimics the effects of acetylcholine and since acetylcholine has been postulated to hyperpolarize the distal processes of the L-neuron, it is difficult to explain why Carbachol depolarizes this cell in the dark. If anything, there should be a membrane hyperpolarization. One explanation for this effect is that the Carbachol (10mM) affected presynaptic cholinergic receptors of adjacent photoreceptors and that these presynaptic receptors subserve lateral inhibitory interactions (Stone, 1982).

If there are lateral interactions between photoreceptors, that are inhibitory or otherwise, pharmacological studies on other aspects of ocellar physiology (besides the local potentials of the photoreceptors and L-neurons) should uncover further evidence of their existence. Since the cholinergic effect on impulse activity had not as yet been studied, a major objective of the research was to find out if there is a cholinergic influence on spike frequency and in effect represents the first pharmacological study on impulse activity in an ocellar nerve.

The addition of GABA (9.7 mM) to the perfusate in one

preparation increased the size of the "on" transient, and altered the shape of the "off" response in the second order cell (Klingman, 1976; Klingman and Chappell, 1978). Picrotoxin and Bicuculline have been shown to be GABA antagonists in a number of preparations (Takeuchi and Takeuchi, 1969; Walker, et al., 1971; Gerschenfeld, 1973; Johnston, 1977; Defeudis, 1977; Ticku and Olsen, 1978). When these agents are individually added to the bathing medium (picrotoxin 1.66 mM, bicuculline 81.6 mM) they a ppear to modify the "off" oscillation of both the L-neurons (Klingman and Chappell, 1978), and the photoreceptors (Stone, 1982). The effects of picrotoxin are partially reversible after washing with Ringer. The L-neuron perfused with bicuculline was lost before washing was attempted (Klingman, 1976).

Bicuculline also affected other aspects of the L-neuron's response. It decreased the size of the "on" transient, lowered the sensitivity of the light response by two log units, decreased the dark noise, and decreased the amplitude of the sustained hyperpolarization (except at the highest stimulus intensity) (Klingman and Chappell, 1978).

Stone (1982) reports that picrotoxin ( 1.6 mM) increased the amplitude and duration of the "off" hyperpolarization of the photoreceptors. There was also a concom-

mitant increase in amplitude of the L-neuron's "off" oscillation.

On the basis of these experiments, it was proposed that there may be a GABA component to the ocellar system. Therefore one of the goals of this study was to determine if GABA has any effects on impulse activity.

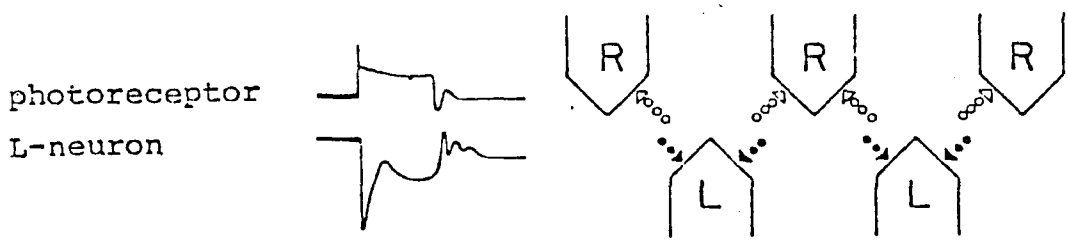
## Synaptic feedback model

The physiological, anatomical, and pharmacological evidence has led to the formulation of a model which proposes that there are local circuits within the synaptic plexus of the ocellus (Chappell and Klingman, 1974; Klingman, 1976; Klingman and Chappell, 1978; Stone and Chappell, 1981; Stone, 1982). The following is an explanation of this model:

In the dark adapted state, the L-neuron releases a certain amount of feedback transmitter from its dendritic terminal. This feedback transmitter may act to facilitate receptor transmitter release. At the same time, the photoreceptor terminal releases a certain amount of transmitter (presumably acetylcholine) which causes the L-neuron's dendrites to hyperpolarize, resulting in a decrease or inhibi-

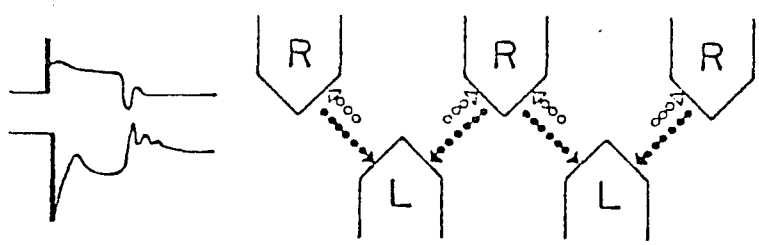
tion of feedback transmitter release.

In the dark, the quantity of both types of released transmitter reaches a steady state or equilibrium. This steady state results in the maintenance of stable resting potentials within both the first and second order cells. The noise often recorded from the L-neurons, in the dark, might represent small variations in the amount of receptor transmitter released (Klingman, 1976).



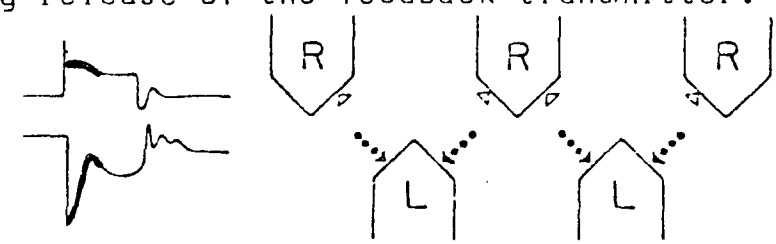

(adapted from Stone, 1982)

At light "on", the receptor cell depolarizes resulting in a large release of transmitter.

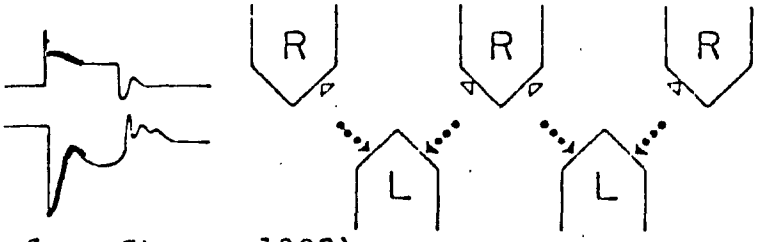



(adapted from Stone, 1982)

Released receptor transmitter further hyperpolarizes the ocellar nerve dendrites, inhibiting release of the feedback transmitter.

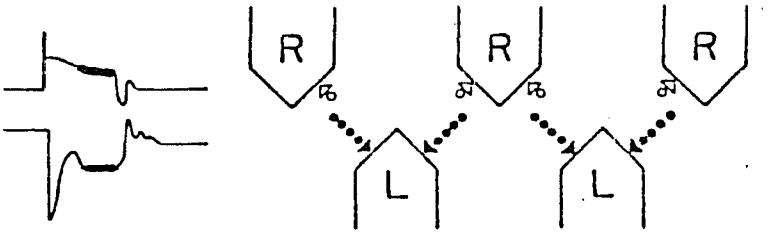



(adapted from Stone, 1982)



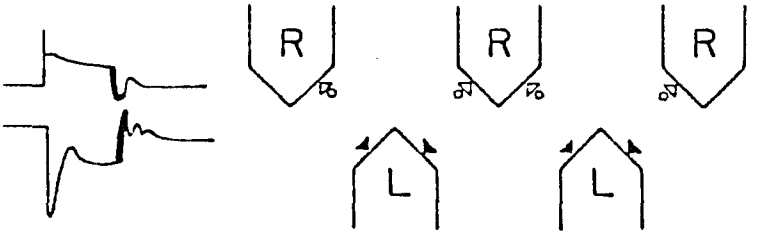

(adapted from Stone, 1982)

Since less feedback transmitter is released, facilitated release of the receptor transmitter is reduced, eventually leading to a new steady state.




(adapted from Stone, 1982)

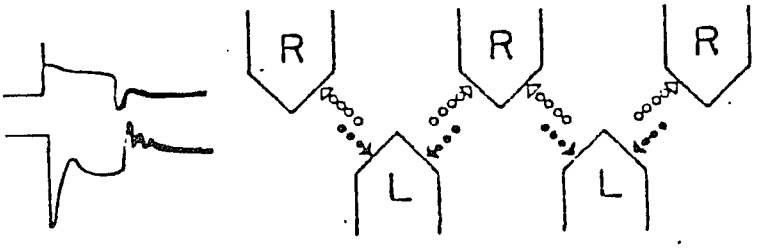
At light "off", the receptor terminal's membrane potential approaches the dark resting potential. This results in a cessation of light-induced release of receptor transmitter.




(adapted from Stone, 1982)

Since the L-neuron was partially hyperpolarized at the steady state level seen in the light, at light "off" it becomes relatively depolarized

resulting in a release of feedback transmitter. The feedback transmitter facilitates release of transmitter from the photoreceptor which results in the negative transient of the L-neuron's "off" response.




(adapted from Stone, 1982)

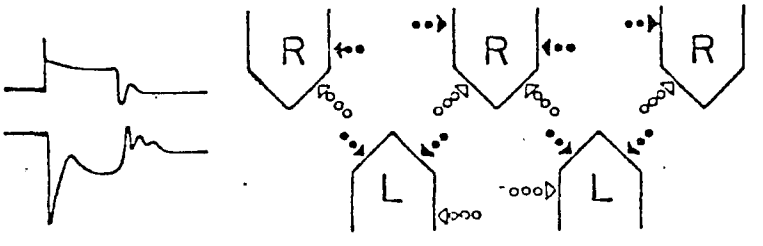
Finally, after a few oscillations in some cases, a new dark adapted steady state is reached.

This thesis deals with a spiking neuron. It is not clear whether this impulse generating neuron is an L-neuron. Some evidence shows that it (the spiking neuron of the lateral ocellar nerve) appears to behave like an L-neuron. At the least it appears to be affected by pharmacological agents that affect the L-neuron. The following arguments assume that the spiking neuron is second order, that it is responding to receptor transmitter in the same fashion as the L-neuron, and that the light intensity-frequency relationship is non-complex.

If there is no feedback and if increasing amounts of acetylcholine decrease ocellar nerve spike frequency in a

graded fashion, then curare, a competitive inhibitor of nicotinic cholinergic receptors (Koelle, 1975b), should shift the (light) intensity-impulse frequency relationship to the right in a parallel fashion with no change in slope (Hollenberg, 1978). Results that show such a parallel shift would not be consistent with the synaptic feedback model, since feedback should result in a more complex alteration of this relationship. However, a complex change involving the slope, the intercepts, or both, as seen in a dose-response experiment, would not rule out an explanation other than feedback to account for such findings (Kerkut and Walker, 1961; Callec and Sattelle, 1973; Sattelle, et. al., 1976). One of the accomplishments of this study was to carry out such an experiment, the results of which are consistent with the model.

Recently, Stone (1982) has expanded the model to include lateral synaptic interactions. She suggests that




(Stone, 1982)

the lateral synaptic interactions between photoreceptors,

whose existence is demonstrated by electron microscopy, are inhibitory. That is, receptor transmitter acts to hyperpolarize neighboring photoreceptors which ultimately may reduce the amount of acetylcholine which reaches the cholinergic receptor sites of the ocellar nerve dendrites.

The presumed lateral synaptic interactions between adjacent L-neurons may be excitatory (Stone, 1982), but there is no hard evidence that this is the case. If they are excitatory, the synaptic release of a small amount of feedback transmitter could lead to a further increase in its own release.

The model emphasizes that the synaptic contacts are in a state of dynamic equilibrium, so that the ocellar system would appear to be geared towards responding to a change in the intensity although to some extent it can respond to the absolute level of illumination.

## Objectives

The primary objectives of this study were:

1. To determine the source of the impulse activity recorded from the cornea of the ocellus.

2. To develop a non-invasive perfusion preparation for the study of ocellar nerve impulses.

3. To study the behavior of the impulse activity under the influence of agents known to affect the photoreceptors and the L-neurons.

4. To compare the behavior of the impulse activity to that of the L-neurons.

# METHODS

## Electrodes

Extracellular recording electrodes were made from 0.25 mm diameter stainless steel wire cut into 7.6 cm lengths. This wire was inserted into the lumen of a number 20 stainless steel Luer Loc syringe needle which was then crimped to hold the wire in place. The tips of the electrodes were sharpened electrolytically using an Ever-eady no. 276 "9" volt battery, at which time the electrodes served as the anode while a carbon rod served as the cathode. The electrodes were rinsed in distilled water and viewed under a dissecting microscope several times during this procedure to check the angle of the shaft. If the tip was too sharp the electrodes bent when pushed up against the animals hard exoskeleton.

The electrolytic solution was prepared in the following proportions:

| | | | | |
|---|---|---|---|---|
| 68 | ml. | $H_2SO_4$ | specific gravity | 1.84 |
| 84 | ml. | $H_3PO_4$ | specific gravity | 1.69 |
| 48 | ml. | $H_2O$ | | |
| 200 | ml. | total volume | | |

Intracellular electrodes were made from 0.8 mm O.D. boron-silica glass capillaries which were pulled on a Narishege horizontal electrode puller for a tip resistance of 50 to 100 megohms when filled with 3 molar cobalt chloride. A silver chloride disc obtained from W.P.I. served as the reference electrode.

## Recording system

Extracellular electrodes were attached to differential inputs G-1 and G-2 on a Grass P15 A.C. preamplifier or inputs A (analogous to G-1) and B on a PAR model 113 preamplifier. The frequency bandpass used was 300 HZ-1 KHZ for monitoring impulse activity and 0.1 HZ - 0.1 KHZ for ERG recordings. The gain was most frequently x1000, but occasionally this was lowered to x100. The preamplifier was capacitatively coupled to the lower channel of a Tektronix 502A Dual Beam oscilloscope. Capacitance values ranged from 0.001 uF to 0.1 uF, depending upon which value gave the best signal to noise ratio for that preparation.

Intracellular recordings were accomplished through a head stage amplifier which was connected to a WPI 707 electrometer amplifier with current injection facilities.

Data was recorded on magnetic tape for later analysis.

The preparation itself was grounded to the chassis of the preamplifier and not to the faraday cage.

## Staining Procedure

Iontophoresis of cobalt chloride into ocellar nerve fibers was accomplished by applying positive 500 nano-amp current pulses through the recording electrode. The duration of each pulse was a half of a second with a 50% duty cycle (Mobbs, et al, 1981). The duration of the cobalt impregnation was from 10 to 30 minutes.

After iontophoresis was complete, the head cavity was flooded with Ringer and one drop of 5% ammonium sulphide was applied for approximately 5 minutes. This was followed by another Ringer wash and a two hour period of fixation in alcholic Bouin. The Bouin fixative was replaced with 50% alcohol and at this time the brain and ocelli were dissected out of the head cavity. Removal of the nerve at this point in the procedure was facilitated by the relative stiffness of the preparation due to the fixative.

It was found that better resolution of the stained processes could be obtained by soaking the preparation

in Timm's silver intensification solution (Tyrer and Bell, 1973; Bacon and Altman, 1976) overnight. Prior to dehydration in graded concentrations of ethanol, the brains were washed in a mixture of one part 10% sodium hydroxide: two parts glycerol:7 parts 70% ethanol (Patterson and Chappell, 1980). This helped to remove most of the surface pigment which could have blocked the visualization of cobalt impregnated fibers. After dehydration the brains were cleared in 100% xylene and mounted whole under Canadian balsam and a cover slip (Mobbs, et.al., 1981).

Photostimulation

The light source in all experiments was a 100 W quartz-iodide bulb operated at 12 volts and 8 amps. A beam of light from this source was focused onto one end of a fiber optic tube. The other end was placed between 1 and 0.5 cm from the vertex at an angle of approximately 45 degrees above the horizontal plane. The light beam was interrupted by an electromagnetic shutter driven by a Uniblitz Model 310B shutter timer control. In the preliminary studies, the frequency and duration of the shutter opening were controlled by a series of Ortec model

4654 delay and duration controls which were in turn triggered by an Ortec model 4653 pulse generator. In later studies, these parameters were controlled by a Digital Equipment Corporation PDP-11 computer using a custom software program which I developed. Lower intensities were produced by using neutral density filters. light at specific wavelengths for spectral sensitivity studies was produced by allowing the light beam to pass through spectral filters before it entered the light pipe. These filters were a Corning CS 737 (wavelength(max) 360 nm, 50% bandwidth 50 nm) and a Kodak no. 85 in combination with a Corning CS5-61 (wavelength(max) 485 nm, 50% bandwidth 30 nm). The filters were selected to approximate the two sensitivity peaks of the ocellar photoreceptors (Chappell and Devoe, 1975) and to duplicate the the experimental conditions used to measure the spectral sensitivity of the median ocellar L-neurons (Mobbs, et al, 1981).

The stimulus was monitored by a phototransistor which was placed in the light path outside of the cage. The output of the phototransistor and a sync pulse from either the Ortec delay and duration controls or the Digital-Out module of the PDP-11 were veiwed on the upper beam of the 502 oscilloscope.

Calibration of the light source was accomplished by

utilizing a United Detector Technology model 40A Optometer photometer. The light pipe was placed 1 cm away from the light collector and measurements were taken directly from the meter and converted to quanta/ square cm/sec when appropriate. The intensity of the unattenuated beam of white light was 640 footcandles.

## Preparation and Dissection

Dragonfly nymphs of the species Aeschna tubiculifera were obtained from Connecticut Valley Biological Supply Company and then raised in the laboratory. As the adults emerged, they were collected and stored for a day to allow their exoskeletons to harden and their pigment to develop.

Intact animals, at least one day old, were attached to a horizontal board with tacky wax. Particular care was taken to immobilize the head. It is very difficult to completely eliminate wing movement in a living breathing dragonfly. However, the wings were Scotch taped to the board in order to inhibit movement as much as possible.

In most experiments, the ocellar nerves and the brain were exposed by placing a thin razor blade at the level of the vertex just above the lateral ocelli and making a horizontal slice through the top of the head. This re-

sults in the removal of the dorsal region of the compound eye. Only a small number of air sacs were removed to allow for adequate visualization of the ocellar nerves without compromising their function. The exposed head was constantly perfused with dragonfly Ringer. The animals appeared to be functioning as normally as could be expected since their wing hinges, antenni, and the eosophagus could be seen moving. For most pharmacological experiments one of the lateral ocellar nerves was removed.

To record action potentials from the median ocellus, the initial exposure of the top of the head was done as follows. The razor blade was placed above the vertex, and the incision was made as previously described. This did not permit visualization of any of the ocellar nerves because the air sacs were not removed, but allowed the head cavity to be perfused with Ringer while the recordings were being made. Lateral ocelli and their nerves were sequentially removed by pushing the tip of a jeweler's forceps through the exoskeleton around the cornea, and pulling the ocellus and its nerve out of the head cavity. By using a dissecting microscope, visualization of the organ within the teeth of the forceps confirmed that the ocellus and its nerve had been removed.

## Solutions

Ionic composition of the dragonfly Ringer's solution (Ringer) was based on an analysis of the hemolymph obtained from the nymphs of the genus Aeschna (Duchateau, et. al., 1953). The Ringer solution consisted of: 134 mM NaCl, 5.4 mM KCl, 3.8 mM CaCl2, 3.0 mM MgCl2, 0.5 mM NaHCO3; and was adjusted to a pH of 7.4 . All drugs were dissolved directly in the Ringer solution (Klingman and Chappell, 1978).

The following is the list of drugs and their sources used in this study: d-tubocurare chloride, United States Biochemical Corporation, Cleveland, Ohio; gamma-amino-n-butyric acid, Sigma Chemical Company, St. Louis, Missouri; cobalt chloride, J.T. Baker Chemical Company, Phillipsburg, New Jersey; tetrodotoxin, Sigma Chemical Company, St. Louis, Missouri.

## Perfusion

All perfusing solutions were applied to the head by

drop perfusion from 30-gauge syringe needles. The needles were soldered to an iron bar with their tips aligned and secured with a narrow strip of heat shrink tubing. One end of the iron bar was secured with an adjustable clamp so that the aligned tips of 5 syringe needles could be lowered into the head cavity of the animal at the same time.

The perfusates were maintained at room temperature outside of the Faraday cage in 100 ml. microdrip chambers (Abbott Company)which were connected to the syringe needles with plastic tubing. The drip rate was modulated via an externally located screw clamp. This made it possible to perfuse the preparation with up to five different drugs or concentrations without opening up the cage.

Before each experiment the perfusing solutions were milked out of the distal end of each plastic tube allowing air to fill up the dead space. The air served as a barrier to prevent inadvertent leakage of the pharmacological agents into the head cavity prior to opening the external clamp.

When changing the perfusing solution the head cavity was gently flooded with the new solution so as to wash out the previous perfusate. The drip rate was then adjusted to approximately 4 drops per minute and maintained at that

rate throughout the experiments.

## Experimental protocol for pharmacological experiments

### Curare

Measurements of spike frequencies were obtained as the concentration of curare in the perfusate was sequentially increased. Control perfusates contained no curare. The data collected at each dose represents the average response from a minimum of six animals. The entire experiment was controlled by the computer using a program called "SPIKES" (see appendix). The program initiated the command to start counting spikes two seconds prior to and throughout a light flash of two second duration. The counting was continued for 26 seconds after light off. Two seconds after the counting had stopped, this sequence automatically repeated. This means that each flash was preceded by a 30 second period in the dark.

Because of the limitations of the equipment the instantaneous spike frequency could not be monitored. Therefore an alternate way was needed to collect the spike counts so that they could be analyzed in a meaningful manner. To do this, time was divided into half-second intervals, with

interval #1 starting 2 seconds prior to the light stimulus. The spike counts were summed for each half second interval and the totals of each interval were stored in a two dimensional array. The first dimension of the array represents the interval number from the start of the counting and the second dimension represents the stimulus number (ie. first flash, second flash, etc.) (see figures 23,24, 25, and 26).

It was possible to monitor the computers ability to count each nerve impulse. This was done by viewing the output of the computer's discriminator which put a dot above each spike which had been counted (figure 17).

In order to insure that the drug concentration had equilibrated at its site of action the animal was left for one hour in the dark, at each dose, before stimulating. This also allowed the animal to fully dark- adapt. The "SPIKES" program was then used to stimulate the preparation with light. For each drug dose, the light intensity was incremented, every three flashes, in whole log units starting from log I = -9 to log I = 0 . Only the counts from the second and third stimuli were used for analysis.

The dark frequency was determined at each drug dose after one hour of dark adaptation. The curve labeled

"DARK", in figure 29, represents the mean frequency in the dark from four out of seven animals. The data was obtained by computer analysis of the tape recorded experiments. For each animal the dark frequency was determined by taking the average frequency for 10 consecutive intervals sometime before the start of the first flash of light.

GABA and Cobalt

A custom softwritten program called "Multispikes" (see appendix), collected the data in much the same way as the "spikes" program, except that the counts from more than one stimulus were summed in each interval. This became necessary because the speed of the computer did not allow for very short interval durations (a necessary prerequisite for instantaneous frequency measurements). In this way atypical responses due to the deviation of individual flashes could be lessened. The program initiated the command to start counting spikes two seconds prior to and throughout a light flash of two second duration. The counting was continued for sixteen seconds after light off. The sequence would automatically repeat until 3, 6, or 9 flashes had occurred. The number of flashes per program run is user

controlled. Data were stored in a two-dimensional array in which the first dimension represents interval number and the second dimension represents histogram number. The duration of each interval was 200 msecs.

For studies on the time course of action of GABA (figures 36,39, and 40), the animal was allowed to dark adapt in Ringer for one hour. Then the animal was subjected to three test flashes at some light intensity. The summed spike counts were then stored and perfusion of the drug at a given concentration was initiated. From time zero, the start of drug application, the computer continued to run and store the summed counts of every three flashes. The result is that every histogram (figure 36) represents the number of impulses per interval from three consecutive stimuli within a time period of 60 seconds.

The investigation of the time course of action of 12 mM cobalt was carried out in the same way except each histogram represents six flashes and counts all spikes over a period of 2 minutes.

For intensity response studies in the presence of GABA (figures 37 and 38), the animal was first dark adapated for one hour, at which time "Multispikes" was used to stimulate the preparation with light. The interval duration was 200 msec. In the presence of GABA, or Ringer

solutions, the light intensity was incremented every six flashes, in whole log units, starting from "dark" to log I=-1. The counts from all six flashes at a given intensity were summed and stored in a histogram . This means that each histogram in figure 37 and 38 represents the total counts of spikes from six consecutive flashes occurring over a period of 120 seconds.

Attempts to reverse the effects of all drugs were carried out by washing the preparation in Ringer for various lengths of time.

Tetrodotoxin

A number of reponses to light stimuli were recorded to demonstrate that the preparation was healthy and functional. The effects of TTX on the impulse activity were then monitored on a CRT while the drug was applied to the animal's head cavity. All experiments were recorded on tape (see "recording system"). Once the impulse activity had disappeared, a DC ERG was obtained from the lateral ocelli to determine if TTX insensitive components, ie. contributions of first and second order cell slow potentials, could still be recorded in response to light thus indicating a viable retina except for the impulse block.

The preparation was washed for an hour in Ringer to attempt to reverse the effects of the drug.

# RESULTS

## Origin of Ocellar Spiking Activity

Extracellular recordings from the cornea of the median ocellus reveal two general types of action potentials, those that are small in amplitude and are light facilitated, and those that are large and light inhibited (figure 4, A and B). All impulses are biphasic and are oriented with an initial negative phase followed by a positive phase (figures 9 and 10). The large action potentials are of 2 sizes, and often infringe upon each other (figure 9, A and D). This indicates that more than one spiking unit is involved.

Following the removal of the left lateral ocellar nerve there was a reduction of the number of action potentials and all of the light inhibited spikes that remained appeared to reflect a single population (figures 4B, 9, B and E). When the right lateral ocellar nerve was removed, no spikes of any size were present (figures 4C, 9C and 9F). Thus it appeared that the lateral ocellar nerves were the source of the spikes. To rule out the possibility that this finding

resulted from damage to either the second order cells and/or the photoreceptors during this procedure, a D.C. ERG was recorded from the median ocellar cup (figure 4D). The presence of component 1 (photoreceptor potential), component 3 (ocellar nerve dendritic potential), and an "off" response in the waveform of the light response implies that the median ocellus and its nerve were undamaged (Ruck, 1961), and is consistent with the idea that the spiking activity is originating from the lateral ocellar nerves.

Differential recordings between the corneas of the right and left lateral ocelli (see figure 5 for recording configuration) also revealed 2 general types of spiking activity, similar to that of the median ocellus, except that in these recordings the larger light inhibited spikes exhibited 2 distinct orientations which often were unequal in size (figures 6,7,9A,9D, and 10). Occasionally it appeared as if a second impulse occurred before a previous impulse had finished (figure 9D). The differential preamplifier to which the corneal electrodes were connected produced an output voltage proportional to the change in potential of electrode G-1 relative to electrode G-2. Except where otherwise indicated, electrode G-1 was placed in the cornea of the left lateral ocellus and electrode

G-2 was placed in the right lateral ocellar cornea (figure 5). In this recording configeration some of these biphasic spikes were oriented with an initial negative phase preceding the positive while others had the positive phase preceding the negative phase (figures 7, 9D, and 10). It should be noted that spikes occasionally suggest a triphasic or monophasic appearance (figures 7 upper trace and 9D). Cutting the median ocellar nerve caused no change in the recorded activity. Sequential ablation of the median ocellus, right and left antennal nerves, optic lobes and cervical neck chain caused no change in the qualitative behavior of the spikes (figure 6).

Upon ablation of the left lateral nerve, the large light-inhibited spikes with the initial positive phase dissappeared. Figure 6 shows that the spikes that were left were still light inhibited. In addition, none of the remaining action potentials occurred before a previous impulse was over. Removal of the right lateral ocellar nerve resulted in the dissappearance of these remaining spikes. From the results of this procedure, it would appear that the spikes which have the negative phase first are originating in the right lateral ocellar nerve and those with an initial positive phase originate in the left lateral ocellar nerve.

To determine if there was a direct temporal correspondence between the occurrence of an action potential recorded from the median ocellus and one recorded from the lateral ocelli, extracellular recordings were made from the cornea of the median ocellus at the same time that differential recordings were made between the corneas of the right and left lateral ocelli (see figure 8 for recording configuration). For each spike recorded from the median ocellar electrode, there was a corresponding spike recorded between the lateral ocellar electrodes, as either the initial positive phase spikes associated with the left lateral ocellar nerve or the initial negative phase spikes associated with the right lateral ocellar nerve. Conversely, every spike that appeared in the differential recording from the lateral ocellar corneas, no matter what its size or configuration, had a corresponding spike of negative/positive orientation in the median ocellar trace (figures 9A, 9B, 9D, 9E, and 10).

Upon ablation of the left lateral ocellar nerve, the initial positive phase spikes dissappeared from the lateral ocellar trace (figure 9B and 9E bottom) along with the corresponding spikes seen in the median ocellar recording (figures 9B and 9E top). When the right lateral ocellar nerve was removed no spikes of any orientation

could be seen in either trace (figures 9C and 9F).

Both the Grass P-15 and the PAR-113 preamplifiers put out a voltage proportional to the potential change at electrode G-1 with respect to electrode G-2. The spikes of the left lateral ocellar nerve are oriented with an initial positive phase when G-1 is in the left lateral ocellar cornea and G-2 is in the vertex or the cornea of the right lateral ocellus (figure 9D and 10B); and they are oriented with an initial negative phase when G-1 is in the median ocellar cornea and G-2 is in the vertex (figure 9D and 10). If differential recordings are made between the corneas of the median (G-1) and left lateral (G-2) ocellus, the spikes are also oriented with an initial negative phase.

In summary, there was a one to one correspondence for every spike seen in both traces, and no significant time lag between a spike seen in the differential recording from the lateral ocellar corneas and the corresponding spike of the median ocellar trace. These results suggest that the spikes seen in the recordings from the cornea of the median ocellus are originating from the lateral ocellar nerves and not the median ocellar nerve as might be concluded from earlier reports (Chappell and Dowling, 1972).

## Intracellular Recordings

Intracellular recordings from the lateral ocellar nerve fibers revealed two categories of neurons, those with and those without spontaneous impulse activity (figure 11).

All non-spiking cells had stable dark resting potentials which ranged from -30 mv to -55 mv. Their response to a 400 msec light flash was an initial transient hyperpolarization, which at an intensity of log I=0 could have a magnitude of -15 mv (figures 11 and 12), followed by a return to a less negative but sustained potential. Sometimes the potential returned to or transiently exceeded the dark resting potential before attaining the sustained level. At light "off", the membrane potential overshot its dark resting level and then gradually returned to its original baseline.

A cell from which impulse activity could be recorded was impaled for 20 seconds (figure 11). Its dark resting potential was -55 mv and the amplitude of its spontaneous action potentials was 30 mv. The action potentials were inhibited by light and exhibited a response latency of at least 10 msec, which is very close to the latency measured for the L-neuron in the median ocellus (Chappell and Dowling, 1972). There was also a transient 5 mv hyper-

polarization which lasted for the first 40 msec of the response followed by a 5 mv positive overshoot of the resting potential (figure 11). The membrane potential remained at this level throughout the period of illumination. At light "off", the membrane potential appeared to rise slightly and the impulses returned with a higher frequency than before the stimulus.

Extracellular recordings revealed impulse activity which appeared to originate in the lateral ocellar nerve, yet these impulses rarely appeared in either intracellular recordings from the presumed second order cells of the median ocellus (Chappell and Dowling, 1972; Stone, 1982; Patterson and Chappell, 1980) and/or similar recordings from the lateral ocellar nerve. It is possible that the lack of impulse activity in the non-spiking neuron was due to cell damage at the time of electrode penetration. To determine if the spiking neuron represented either another class of nerve cells or was in fact a "healthy" L-neuron, an extracellular recording between the corneas of the right and left ocelli was carried out at the same time that an intracellular electrode was penetrating a process in the right lateral nerve. The result was that an L-neuron like response could be recorded from the nerve fiber at the same time that spiking

activity was observed in the extracellular trace (figure 12). The process from which the intracellular response had been obtained was stained with cobalt and shown to have a dendritic tree within the right lateral ocellar cup (figures 2A and 2B). It can be seen that the fiber appears to be going to the ipsilateral protocerebrum and to end in the area near the ventrolateral dendritic complex (Mobbs, et. al., 1980) (figure 2A). Since the left lateral ocellar nerve had been severed, these spikes could only have been coming from the right lateral ocellar nerve. An additional observation that is consistent with this idea is that, as the microelectrode was advanced into the nerve, the spike rate could be seen to increase. This may have resulted from the increased tension put on the nerve fiber as the electrode entered it.

These results suggest that there is another class of neurons within the lateral ocellar system, that may be either second or higher order, but unlike the L-neuron, has spontaneous impulse activity which is inhibited by light.

## Occurrence of Small Amplitude "On" Spikes

The smaller action potentials, mentioned in a previous section of these results, were facilitated by light (figures 4 and 6). This is consistent with findings from previous studies (Kondo, 1978; Rosser, 1974; Chappell and Dowling, 1972). Several investigators have suggested that these impulses carry information from the brain to the ocellus (Kondo, 1978; Rosser, 1974). In the preparations in which they were seen, their frequency would decrease within a few hundred milliseconds after the start of the stimulus (figure 4B). Also, the spikes would sometimes appear as a burst at light "off" as well as at light "on", which along with their rapid adaptation to light suggests that their activity is phasic in nature (figure 18 bottom two traces). A few small spikes occasionally appeared spontaneously in the dark (figure 18 top trace).

In a large number of preparations this light facilitated impulse activity would completely dissappear for no apparent reason. This erratic behavior precluded any detailed study of them. However, since no impulses could be seen after the ablation of both lateral ocellar nerves (figures 4 and 6), it seems reasonable to suggest

that they originate from fibers traveling within these nerves.

## Behavior of Afferent Spikes

The large light-inhibited action potentials of the lateral ocellar nerve occur with an average frequency of twenty-two spikes per second after at least one hour of dark adaptation (figures 6 middle, 18 top, and 29). Under this condition the firing rate was arhythmic (figures 6, 9A, 9B). The response to a two second, light stimulus of low intensity was a reduction in frequency. The greatest reduction occurred during the first 200 to 400 milliseconds, after which time the spikes increased in number until a new steady state rate was achieved which was less than the firing rate in the dark (figure 18). In most preparations, a stimulus magnitude of log I=-5 resulted in complete inhibition, for the two second duration of the stimulus (figures 13, and 18).

At light "off", two types of spiking behavior were characterized. In most preparations, at the time that the stimulus is turned off a burst of activity is seen which decreases with time eventually returning to the dark

rate (figure 18). This gradual return to dark rate can take longer than 30 seconds when the stimulus is very bright. A few preparations show a single or short burst of spikes at light "off", followed by a quiet period or decrease in firing rate which can last up to a few seconds. The frequency of impulses gradually returns and eventually reaches the preflash frequency. While there was considerable variation in the behavior of the spikes from preparation to preparation, any given preparation showed consistent behavior throughout an experiment.

A typical frequency response curve, for the last 1.5 seconds of a 2 second flash, is seen in figure 13. Note that complete inhibition of impulse activity occurs at log I=-5. In Ringer the threshold of a typical response to light occurs between log I = -9 and -8 (figures 18, 33, and 34).

The spectral sensitivity studies on ocellar nerve impulse activity showed that the spike frequency has a lower threshold of inhibition for green light (485 nm) than it does for ultraviolet light (360 nm)(figure 14). The stimulus intensity necessary to elicit a threshold response was approximately 1 x 10E8 quanta/cm2/sec for green light and 1 x 10E10 quanta/cm2/sec for UV light. However the response threshold of the impulse activity

in the lateral ocellar nerve preparation studied, was one order of magnitude more sensitive to green light than that reported for median ipsilateral L-neurons, while the thresholds to UV light were comparable (Mobbs, et al., 1981).

Figure 33 suggests that the maximum response to light is reached somewhere between the intensities of log I=-4 and -5. The response appears to decrease in magnitude for higher intensities. Hyperpolarizing responses to light, recorded from median ocellar L-neurons, also reach a maximum amplitude at some intermediate light intensity. The magnitude of the response then decreases as the level of illumination is increased beyond this point (Chappell and Dowling, 1972).

These results show the clear resemblance of the behavior of the spiking neuron to that of other second order cells of the dragonfly ocellus ( see Discussion: Behavior of Spiking Activity). Also, they suggest an increased sensitivity of the impulse activity to light when compared to the slow potential changes recorded from the L-neuron (Chappell and Dowling, 1972; Mobbs, et. al., 1981).

## Pharmacology

### Tetrodotoxin

The effects of 0.3E-6 M TTX on spike activity on two preparations occurred within seconds (figures 15 and 16). Initially the amplitude of the extracellularly recorded action potentials decreased without any comparable change in the background noise. Since the recording electrodes were AC coupled to the preamp, this diminution of amplitude probably represents the decreased rise time of each spike due to the action of the drug. By the end of the first minute all impulse activity had dissappeared from the trace. The presence of a healthy ERG (figure 16 bottom) is consistent with the notion that the slow responses of the photoreceptors and ocellar nerve dendrites were unaffected by the drug. The effects were not reversible even after one hour of washing in Ringer.

### Curare

In the absence of drugs, light stimulation caused an intensity dependent decrease of the interflash spike fre-

quency as compared to the spike frequency in the dark (figure 18, 23, and 30). The interflash spike frequency is defined as the impulse frequency measured 30 seconds after a light flash. The data were plotted on a PROPHET graphics terminal and analysed using the statistics program TEST LINES. The program carries out an analysis of covariance for multiple regression, determines whether the slopes are significantly different, and calculates for any given line whether Y is independent of X . The points chosen to calculate the slope were done so by visually determining the straight part of the curve. In Ringer the null hypothesis H0: Interflash frequency is independent of the light intensity at the previous flash, was rejected at $p<0.01$, $n=7$ (figure 30).

The seven statistically analyzed preparations continued to have spiking activity throughout the duration of the experiment. Preparations in which impulse activity disappeared were not included. The data obtained from the seven preparations were averaged for a given drug dose during or after a two second light flash at each intensity. The parameters thus obtained for a given experimental condition were then plotted using a PROPHET program called MAKE GRAPH.

At low concentrations (50 uM) curare decreased the

interflash spike frequency for all light intensities (figure 19,29, and 30). However, it had no significant effect on the spike frequency when the animal was allowed to dark adapt for one hour (figure 29 and 22A). To determine if this effect was due to the drug or merely the increased time in the dark during which the preparation was perfused, one animal was perfused with Ringer for a second time instead of with 50 um curare (figure 28). The second intensity-response series was similar to the previous series in that preparation. In fact, the second series appeared to have a slightly higher frequency between flashes. These results are consistent with the idea that at this dose, the effect of curare is to decrease the rate of dark adaptation. More accurately, it appeared to take a longer period of time for the spike frequency to return to its dark-adapted level.

At this drug dose the null-hypothesis H0:Interflash frequency is independent of the light intensity of the previous flash, was rejected at $p<0.01$, $n=6$ (figure 30).

When the concentration of curare in the perfusate was increased to 100 uM, instead of a further decrease in the interflash frequency, the average of 7 preparations showed an increased frequency which approached control values (figures 29 and 30). The independence of interflash

spike frequency to the light intensity at the previous flash was tested and shown to be true at $p>0.05$, $n=7$. So it appears that, at this dose, the spike firing rate between flashes is intensity independent. Figure 24 is a series of histograms showing ocellar nerve spike frequency, recorded from a single preparation, before, during, and after a two second light stimulus. In this animal interflash spike frequency had not increased to the values recorded during perfusion with Ringer as seen in figure 23. However, the figure does show that the spike frequency seen 30 seconds after a light flash is independent of the stimulus intensity.

At the higher concentrations of drug, the interflash spike frequency increased in a dose dependent manner (figures 20, 21, 25, 26, and 29) and remained essentially intensity independent (figure 30), $p>0.05$, $n=7$. There was also a significant dose dependent increase in the dark adapted spike frequency, but the slope of this increase was less than the slope of the mean of the interflash frequencies, so that the two lines representing the dark frequency and the interflash spike frequency are converging (figure 29). These results have uncovered a previously unreported effect of low doses of curare on a physiological response to light in the dragonfly ocellus.

What is particularly interesting is that this effect occurs at some time after the stimulus.

The range of the physiological response to light, from threshold to saturation, occurs between log I=-8 and log I=-5 (figures 18, 23,and 33), so that the normal range of the immediate response to light covers 3 orders of magnitude of intensity. Low concentrations of curare do not appear to significantly affect this range but they do change the slope of the intensity response relationship at $p<0.01$, $n=7$ (figures 32 and 34). However, doses of curare of 500 uM or higher increase the range of the light response to over 5 orders of intensity (figures 31, 33). Also, increasing concentrations of drug shift the intensity-response curves of the impulse activity in parallel (figures 32 and 34). The null hypothesis H0:The slopes are not different (excluding Ringer) was accepted at $p>0.05$, $n=7$.

In the presence of low concentrations of this drug, the impulse frequency in the light appears to be less than control frequencies (during illumination) measured in Ringer (figures 19 and 31). At a dose of 100 uM the frequency at all intensities increased from the 50 uM curare level, reapproaching the control values (figure 31). This is consistent with the observation that the

spike frequency has a dose dependent character which, above 50 uM curare, increases proportionately with increasing concentration.

Above 100 uM it appears that with increasing intensities of light, the average spike frequency (n=7) during stimulation reaches a non zero level at which there is no further significant decrease (figures 31 and 33). This final plateau level appears to be dose dependent and may reflect the effect of the drug's on the baseline frequency measured in the dark.

When individual experiments were examined, in all cases the lower doses of curare decreased the slope of the line analagous to the 50 uM curve seen in figure 31 and lowered the stimulus threshold at which a response could be observed. Additionally, in every preparation, increasing the concentration of curare above 50 uM resulted in a dose dependent shift of these curves to the right. This shift is similar to what one would observe after the application of a competitive inhibitor.

If one looks at the difference in spike frequency just prior to and just after a light is turned "on", as a function of stimulus intensity, it can be seen that the difference reaches a maximum somewhere between log I=-5 and -4 and then begins to drop off as the intensity is furth-

er increased (figure 33). This intensity-response relationship is similar to that of the hyperpolarizing response reported for the L-neuron of the dragonfly median ocellus (Chappell and Dowling, 1972).

A low curare concentration appears to slightly lower the maximum difference but it does not significantly alter the shape of the curve. Higher doses abolish the decrease seen after log I=-4. At concentrations above 100 uM, the curves appear to shift, in parallel, to the right. This is consistent with the notion that curare is acting as a competitive inhibitor of the receptor transmitter.

The effects of curare were partially reversible (figure 27). In all cases washing in Ringer for an hour resulted in a decreased interflash and dark-adapted frequency and a diminished response to light.

## GABA

10 mM gamma-amino-butyric acid (GABA) was added to the perfusate while the preparation was repetitively stimulated with a two second flash of light (log I=-5) every 18 seconds. Over the duration of perfusion the light response changed in character. The initial effect was that there was no longer complete inhibition of impulses during light

"on" (figure 35). By 4 minutes, the frequency during the sustained portion of the light response was close to its interflash value and the off burst was noticably diminished. After 12 minutes it seemed as if the appearance of spikes was facilitated by light. As for the spontaneous impulse activity, the interflash frequency was generally lower after the application of GABA. At 23 minutes, the interflash frequency increased for a period of about 5 minutes. After this transient increase the frequency between flashes gradually decreased until it was almost zero at 56 minutes after the start of the drug perfusion. At this time, light stimulation resulted in a burst of spikes which reached its maximum frequency just after light "off".

A series of histograms showing the number of spikes per interval after time zero demonstrates that action potentials appear to be facilitated by light in an intensity range between threshold log I=-6 and log I=-3 (figures 36 and 37). Another interesting finding is that as the intensity of illumination was increased from threshold, the maximal spiking activity was shifted towards light "off" (figure 37). At log I=-2.5 and greater, impulse activity was not increased during illumination, but large intensity dependent "off" responses occurred, which were character-

ized by a burst of impulses at light "off" that gradually decreased in frequency with time as compared to the response measured in Ringer at the same stimulus intensity (figure 36).

The effects of GABA were partially reversible by washing in Ringer (figure 38). The sequence of the restoration of the original response was the reverse of that seen during the time of drug perfusion.

## Cobalt

In an effort to determine the spike firing rate in the absence of synaptic transmission, a nonspecific inhibitor of synaptic vesicle release was added to the bath. Application of 12 mM cobalt for 18 minutes caused the disappearance of the action potentials (figure 39). The sequence of pharmacological effects started with an increase in interflash spiking rate followed by a gradual decrease in frequency until no impulse activity was present. This effect was partially reversed after 13 minutes of washing. An application of 30 mM cobalt irreversibly eliminated all impulse activity.

# Discussion

## Localization of ocellar impulse activity

In the dragonfly, action potentials can be recorded between an extracellular electrode placed in the cornea of the median ocellus and a second electrode placed in the animal's vertex (Chappell and Dowling, 1972). The size and behavior of these spikes falls into two broad categories, those that are large in amplitude and inhibited by light and those that have smaller amplitudes and are light facilitated (figure 4B).

Extracellular lateral ocellar nerve recordings show action potentials that exhibit these same two size and behavioral characteristics (figure 6)(Ruck, 1961; Rosser, 1974; Kondo, 1978;), and previous investigations have provided evidence that the large spikes are afferent and the small spikes efferent (Kondo, 1978)(see Background Information: Impulse Activity). In this study, it was found that as long as one lateral ocellus and its nerve is intact, corneal recordings of both types of spikes can be made, even after the ablation of all other known photosensitive organs. The finding that the frequency of the

large spikes is photosensitive under these conditions is consistent with the notion that they are afferent in nature, but the observation that the frequency of the small spikes still change in response to light requires some discussion.

If the small spikes carry afferent information from the lateral ocellus to the brain, then the implication is that the fibers in which they propagate receive synaptic input at or near the ocellar cup. This conclusion is not in agreement with some of the evidence. Kondo (1978) was able to record small light modulated spikes from the proximal stump of a severed ocellar nerve, with the compound eyes and other two ocelli intact. From his results one might be lead to believe that the frequency of the small spikes is modulated peripherally by neurons located in one of the other ocelli or compound eyes. However, if this were so, then after the extirpation of all other photosensitive organs, there should be no light induced frequency change of the small spikes in the remaining lateral ocellar nerve, and this is clearly not the case (figure 6).

Since under these conditions, the only light sensitive cells that are left are located in the remaining lateral ocellus, an alternate explanation for the photo-

modulation of the frequency of the small spikes could be that the fibers that carry them are receiving information in the brain from afferent ocellar nerve fibers. This means that the small spikes are carrying efferent information "back" to the ocellus. The idea that the small spikes are efferent in nature is in accord with the findings of the previous studies and opens up an interesting possibility of the existence of a "long" feedback loop.

Ruck (1961) showed that light modulated action potentials could be recorded from the lateral ocellus with a corneal electrode and he assumed that these impulses originated from the lateral ocellar nerve of the ocellus from which he was recording. Since the median ocellus also has a nerve, although much shorter than the two lateral nerves, it was assumed that the spikes recorded from the cornea of the median ocellus were coming from the median ocellar nerve (Chappell and Dowling, 1972).

The results of my experiments suggest that the spiking activity recorded using a corneal electrode in the median ocellus does not represent spiking activity from the median ocellar nerve. For instance, during recordings between the lateral ocellar corneas, removal of the median ocellar nerve did not appear to affect the impulse activ-

ity. However when electrical activity was recorded from the cornea of the median ocellus (figure 4), impulse activity was present as long as one of the lateral ocellar nerves was intact. As soon as both nerves were removed the impulses disappeared, yet the median ocellus and its nerve were untouched. In order to detect damage to the median ocellus, an ERG was recorded at the end of the experiment (figure 4D). The ERG showed that components 1,2,3 and the "off" response (Ruck, 1961; Chappell and Dowling, 1972) were present. These components represent the electrical contributions of the photoreceptors and second order cells to the recorded waveform (Ruck, 1961). A "healthy" ERG indicates that both the first and second order cells of the median ocellus were functioning normally. Therefore, it was unlikely that the inactivation of impulse activity was due to cellular damage within the ocellar cup as a result of the ablation.

Since simultaneous recordings of impulse activity from the median and lateral ocelli show a one to one correspondance (figures 9 and 10) it may be possible that the median ocellar nerve could act as an electrode from which one could record action potentials originating in the lateral ocellar nerve.

Alternatively, the "reference" electrode, by way of the extracellar fluid, could be making contact with any number of light modulated spiking neurons located throughout the head cavity. If the corneal electrode were electrically isolated from the "reference electrode" by the ocellar sheath, then the corneal electrode could serve as the reference and the electrode in the vertex could act as the active electrode. Evidence has been published that the sheath of the cockroach ocellar cup acts as a high resistance pathway to current flow since at light "off" a potential difference exists across it (Ruck, 1966). This sheath could act the same way in the dragonfly ocellus.

Although impulse activity has been recorded from both the cornea of the lateral ocellus (Ruck, 1961), and directly from the lateral ocellar nerve (Ruck, 1961; Rosser, 1974; Kondo, 1978), no conclusive evidence has been published that the spikes recorded at the cornea are in fact coming from that ocellar nerve. Indeed this study shows that one can record nerve impulses from one ocellar cornea that in fact originate from a different ocellar nerve.

There is other evidence that action potentials are not normally present in the median ocellar nerve. In intracellular recordings made within the ocellar retina

from the dendrites of median ocellar nerve axons, and in recordings obtained from L-neuron proccesses within the brain, action potentials are rarely seen (Chappell and Dowling, 1978; Klingman and Chappell, 1978; Patterson and Chappell, 1980; Mobbs, et. al., 1981; Stone, 1982). The rarity of the event suggests that its occurrence may have been due to cellular damage. When this study was first undertaken, a number of months were spent trying to obtain extracellular recordings from the median ocellar nerve using a suction electrode. This technique had already been used successfully to record action potentials from the dragonfly lateral ocellar nerves (Rosser, 1974; Kondo, 1978). However, in no instances could it be conclusively determined that spikes were recorded from the median ocellar nerve, even though they could be obtained from the lateral ocellar nerve.

To answer the question as to whether or not intracellular recordings by their nature damage the cell during penetration and therefore prevent the cell from firing spikes,the following experiment was performed.

Within the lateral ocellar nerve an intracellular recording was obtained from a non-spiking neuronal process simultaneously with an extracellular recording of the ocellar nerve impulse activity (figure 12). The

non-spiking cell was subsequently stained and shown to be an ipsilateral L-neuron (figure 2). The significance of this experiment is that we have identified a typical L-neuron on the basis of its intracellular response and anatomy. We have demonstrated that there were no impulses recorded from this cell at the same time that normal extracellular responses were obtained. Consequently, in this case at least, it could not be said that the absence of spikes in the intracellular recording was due to the injury of the spiking cell whose responses could still be recorded extracellularly. If indeed the L-neuron could produce spikes, they are not the spikes normally recorded with an extracellular electrode.

The spiking unit recorded from intracellularly (figure 11) was probably from an off-type spiking unit (Ruck, 1961). It's activity corresponds to that recorded extracellularly from the lateral ocellar nerve, although it could not be conclusively shown that this spiking neuron was in fact responsible for the extracellular impulse activity. The fact that an intracellular recording of a spiking cell could be obtained from the lateral ocellar nerve is consistent with the idea that a cell that exhibits spontaneous impulse activity at least

sends a process into this ocellar nerve.

The question arises as to why impulses are recorded in the lateral ocellar nerve but not in the median. It has been shown in a number of preparations (Stuart and Oertel, 1978; Pearson, 1979; Shaw, 1979) that slow potentials are sufficient to transmit information over short distances. Since the median ocellar nerve is relatively short, perhaps action potentials are not necessary for ocellus-to-brain communication in this structure. Whereas the lateral ocellar nerves, which are approximately three times the length of the median nerve in Aeschna, Anax (Chappell, et. al., 1978), and Libellula (personal observation) may need regenerative potentials to maintain effective ocellus-to-brain communication in some of its neurons.

Although the intracellular recording from the non-spiking cell in figure 12 was made approximately midway between the ocellar cup and the brain, the amplitude of the transient part of the light response was -15 mv at a stimulus intensity of log I=0. This is very close to the amplitude of this portion of the light response of the ocellar nerve dendrites recorded intracellularly directly in the median ocellar cup (Chappell and Dowling, 1972; Klingman and Chappell, 1978). Similarly,

the spiking neuron, recorded intracellularly in this region of the lateral ocellar nerve, exhibited a 5 mv hyperpolarization for the transient portion of the response. Consequently, it is possible that both impulse and slow potential information can reach the brain in the spiking neuron, although this study deals with the impulse activity exclusively. It should be mentioned that neurons which carry both impulse coded and analog coded information have been reported in the nerve which carries information from the lobster oval organ, a mechanoreceptor (Pasztor and Bush, 1982) and in the brain of the barnacle (Stuart and Oertel, 1978; Ozawa, et. al., 1975).

## Threshold and spectral sensitivities

It has been reported that an ERG can be elicited at stimulus intensities as small as 10E-5 footcandles in the dragonfly ocellus (Ruck, 1961). At that time, the investigator felt that it was sufficient merely to report that the spike activity was more sensitive than the ERG, without actually measuring it. Other publications concerned with dragonfly ocellar impulse activity have not addressed themselves to responses in the threshold range (Kondo, 1978; Rosser, 1974). The results of this investigation demonstrate that ocellar nerve impulse activity was effected by a light stimulus with an intensity as low as log I=-8 (figures 33 and 34). Since in this study, a stimulus intensity of log I=-8 is equivalent to 6.4 x 10-7 footcandles, it appears that the light response of the giant afferent is at least 1.5 log units more sensitive than the ERG measurments. This is the most accurate approximation of the spiking neurons response threshold using a quartz halogen lamp as a light source. Using the same type of light source, intracellular recordings revealed the L-neuron's response threshold to be 1.4 x 10-3 footcandles (Chappell and Dowling, 1972).

Although careful experiments for the accurate determination of threshold have not been carried out on the L-neuron, my results are consistent with the idea that the spiking neuron has a higher sensitivity to light than can be determined on the basis of recorded slow potentials in any individual ocellar nerve dendrite.

## Spectral studies

In the early part of the nineteenth century it was discovered that humans are relatively more sensitive to light of shorter wavelength at or near the visual threshold than they are at greater intensities of illumination (Purkinje, 1819). This increased sensitivity to shorter wavelength light, as the level of illumination approaches threshold, is called a Purkinje shift. In studies of spectral sensitivities of dragonfly ocellar neurons as determined by intracellular recordings, it has been reported that a reverse Purkinje shift exists for half of the photoreceptors examined (Chappell and DeVoe, 1975). The phenomenon is also present in the L-neurons (Mobbs, et. al., 1981) of the dragonfly's median ocellus. A reverse Purkinje shift is defined as ..."a decrease in the relative sensitivity of the dragonfly ocellus in the UV

(short wavelength) end of the spectrum as the level of illumination decreases ..."(Mobbs, et al, 1981).

In a single preparation, it was shown that ocellar nerve impulse activity exhibited a greater sensitivity to green light (485 nm) than it did for UV (360 nm) light from threshold to complete inhibition. An analysis of covariance of the intensity response curves plotted for the two wavelengths showed that the slopes were parallel at $p>0.05$ (figure 14). This indicates that unlike the non-spiking ocellar neurons, the spectral sensitivities did not shift. What is significant about this finding is that the impulse activity appears to preserve the spectral information collected in the ocellar cup. In other words, the greater sensitivity that some of the ocellar photoreceptors have to green light, at low intensities of light, is not lost at higher levels of integration within the ocellar retina.

## Behavioral similarities between the L-neuron and the spiking neuron

Ruck reports that at the cellular level there appears to be no fundamental difference in the organization of the lateral and median ocelli (Ruck and Edwards, 1964), and

he has shown that the ERGs recorded from the median (Ruck, 1958) and lateral ocelli (Ruck, 1961) are identical.

The hyperpolarizing responses recorded from the lateral ocellus (figures 11 and 12) show close similarities to those responses of the median ocellus (Chappell and Dowling, 1972; Klingman, 1976; Stone, 1982). In the median ocellus, using procion staining techniques, cells which produce a depolarizing light response have been identified as photoreceptors and the processes which produce the hyperpolarizing response to light have been shown to be the dendrites of the fibers of the ocellar nerve (Chappell and Dowling, 1972; Patterson and Chappell, 1980).

Since the ERG reflects the overall electrical response of the cells of the ocellar retina (Ruck, 1961; Ruck, 1966), and since the neurons of the median (Chappell and Dowling, 1972) and lateral ocellus appear to have similar intracellular responses, it seems reasonable to assume that all three ocelli have a similar physiology.

Besides the increased sensitivity to longer wavelength light at low illumination levels, this investigation has uncovered a number of other similarities between the light response of the ocellar spiking neuron and those of the L-neuron.

The shape of the histograms of spike frequency in the presence of a dim light resembles the intracellular responses of lateral ocellar non-spiking neurons. The frequency histogram shown in figure 40 was generated using the "Multispikes" program at the light intensity log I=-7 (see Methods:Experimental protocol for pharmacological experiments, GABA and Cobalt). It can be seen that the greatest decrease in spike frequency occurred during the first 400 milliseconds of the light response. During the remainder of the light stimulus the frequency continued to rise, never quite reaching the "dark" level. Many light responses show the spike frequency reaching a new steady state during the last second of a two second flash which is less than the frequency in the dark (figure 23, log I=-7). This is analogous to the description of the light response of the non-spiking neuron seen in figure 11B, only here it is the membrane potential which is changing rather than the frequency. At light "off" one can see a slight rise in impulse frequency and a slight fall in membrane potential of the non-spiking neuron. The similarity between the shape of the frequency histogram and the waveform of the non-spiking cell's membrane potential is to be expected if 1) impulse frequency is proportional to membrane potential (Katz,

1950) and 2) the spiking neuron is receiving the same synaptic input from the photoreceptors as is the L-neuron or is postsynaptic to the L-neuron. Although the latter point is presently not known, the results as presented are consistent with this idea.

The shape of the curve expressing the change in impulse firing rate due to light (figure 33 in Ringer) and the intensity response relationship for median ocellar nerve dendrites (L-neuron) as determined from intracellular recordings (Chappell and Dowling, 1972) are also distinctly analogous. It is difficult to compare the values of the curves on a point for point basis because of the differences in calibration and delivery of the optical stimulus between the two studies. However, both curves reach a maximum before the highest intensity stimulus is reached, and then they begin to diminish in response amplitude.

Dowling and Chappell (1972) have suggested that the mechanism for the saturation of the ocellar nerve dendrites response curve may result from the rich synaptic neuropile which could mediate lateral and feedback interactions. This might also be the explanation for the saturation and diminution of the change in impulse firing rate due to light. However, this effect on

impulse frequency change may merely be due to the fact that impulse activity is completely inhibited by light whose intensity is greater than log I=-5 or log I=-4 (figure 13). Since there was an intensity-dependent decrease in interflash spike frequency (measured 30 seconds after a light flash) (figure 30), the frequency just before the second or third light flash at a given intensity (see methods) would be less than the interflash frequency at the previous intensity. Above the stimulus intensity of log I=-4 there is complete inhibition of impulses, but as the intensity is increased above that level the frequency prior to stimulation is diminished. In other words the preparation is going from some interflash frequency to zero frequency. Since the interflash spike frequency is decreasing with increasing stimulus intensity, the change in spike frequency due to light would appear to diminish.

The similarities in behavior can be accounted for if one postulates that the spiking neuron is a second order neuron receiving information directly from the photoreceptors, or is third or higher order, its response being mediated through the L-neuron.

A neuron analogous to the spiking neuron of the dragonfly ocellus has been found in the brain of the

barnacle (Stuart and Oertel, 1978). This neuron appears to have a connection with this animal's ocellus and is named an Amplifying cell or A-cell. The properties of the A-cell suggest that it is third order, that it fires tetrodotoxin sensitive spikes, that it is more sensitive to light than lower order cells, and that its intensity-response range is narrower than the identified second order neuron of that system.

## A Model: spike firing between two thresholds

A model which could serve as a framework to explain some of the experimental findings is one which presumes that the spiking neuron is second order to the photoreceptor. The impulse generating cell would receive inhibitory cholinergic input from the photoreceptor. It would also receive excitatory GABAnergic input from other ocellar nerve dendrites which behave in a manner similar to the L- neuron of the median ocellus. The idea that there are lateral synaptic interactions between L-neurons and between photoreceptors has been suggested previously by Stone (1982).

The spiking neuron would also have two spike firing thresholds. A low threshold below which no impulses are generated, and a high threshold above which no impulses are generated (figure 41). A neuron with such a mechanism has been identified in the stomatogastric nervous system of the lobster Homarus gammarus (Robertson and Moulins, 1981). The depolarization of this neuron above a membrane potential of -30 mv results in the inhibition of impulses. A hyperpolarization greater than -60 mv also results in spike inhibition (Robertson and

Moulins, 1981).

In the absence of synaptic transmission, the membrane potential could be below the lower spike generating threshold and this is consistent with the experimental finding that ocellar nerve preparations perfused with 12 mM cobalt did not fire impulses. If this is the case, then, perhaps GABAnergic input is necessary for spike initiation. In the dark, under normal physiological conditions, a small amount of inhibitory input from the photoreceptors and a greater amount of excitatory input from the ocellar nerve dendrites would impinge upon the dendrites of the spiking neuron, summate at the site of spike initiation, and thus bring the membrane potential, at this site, above the lower threshold.

In the light, the double effect of a lot of inhibitory transmitter and very little excitatory transmitter would result in an inhibition of activity.

At light "off", the dual action of the absence of inhibition and the release of excitatory transmitter would result in a sudden increase in frequency which would subside as GABA facilitated release of receptor transmitter made the membrane potential more negative.

In many preparations, the spiking neuron fired a single spike or burst of spikes at light "off", which was

followed by a period of inhibition sometimes lasting for seconds. The impulse activity would then return after a period of about a second, at a higher rate than before the stimulus. After a number of seconds, depending upon the stimulus intensity, the frequency would gradually return to its preflash values. A conceivable sequence of events that might be consistent with these experimental findings is that at light "off", the presumptive GABAnergic input onto the spiking neuron depolarized its membrane above that of an upper threshold to firing. The rapid decrease in membrane potential past the higher threshold might allow the single or short burst of spikes followed by the inhibition. As the membrane potential dropped, possibly due to a GABA facilitated acetylcholine release, the potential would drop below the upper threshold and the neuron would begin to fire impulses at a higher frequency than its preflash rate.

Although high doses of GABA can inhibit impulse activity, during the time course of drug action, it can be seen that the impulse firing rate first increases in the dark and the light before complete inhibition takes place (figure 35). Also right after the start of the wash, one finds the firing rate in the dark coming back at a much higher frequency than prior to the start of drug

perfusion (figures 35 and 38). This is consistent with the notion that low concentrations of GABA increase spike frequency, and that washing causes the subsynaptic GABA concentration to decrease with time. A dose response study on the effect of increasing concentrations of the drug on impulse frequency would be an interesting test of the model in the future.

When an intensity-response series was done in the presence of GABA, although no impulse activity was observed in the dark, action potentials were seen during the time of illumination. One explanation for this might be that since light stimulated release of the acetylcholine would tend to make the membrane potential more negative, it could bring the membrane potential below the upper impulse firing threshold and thus increase the probability of firing an action potential(figure 41). This could account for the burst of impulses which are seen during low and intermediate intensity illumination, the appearance of spikes during the interflash period after high intensity light stimulation, and their subsequent inhibition during the light. It might also account for the shift in the occurrence of the maximum GABA-induced impulse activity seen during the light as the intensity is increased up to log I=-3.

Since the magnitude of a post-synaptic response is proportional to the concentration of transmitter (Katz, 1962), intracellular recordings from L-neurons suggest that a larger amount of transmitter is released from the photoreceptors at the beginning of a light response relative to the amount released during the sustained portion of the light response. That is, one can see an initial transient hyperpolarizing wave at the beginning of the response which is larger in magnitude than the sustained hyperpolarization seen after the first few hundred milliseconds after light "on". The release of receptor transmitter at the beginning of a low intensity light stimulus could account for the spike burst seen at light "on" in the presence of GABA (figure 37), since this coincides with the occurrence of the transient hyperpolarization of the response. If transmitter release then returned to some sustained level so that membrane potential returned to a level above the upper threshold, spiking activity would more likely be facilitated during the initial part of the light stimulus. As the stimulus intensity was increased, the initial transient wave would drop the membrane potential below the lower threshold at the beginning of the light flash so that no impulses would appear at that time, but the sustained hyperpolarization,

which is lower in magnitude than the initial transient wave, could bring the membrane to a potential between both thresholds thus allowing the neuron to fire impulses. At the highest intensities both the transient and sustained hyperpolarization might bring the membrane potential below the lower threshold for the entire period of illumination resulting in complete inhibition.

If there is an increased concentration of receptor transmitter present during the time between flashes of a bright light (when the preparation is perfused with GABA) then this might account for the appearance of impulse activity during the interflash period and its subsequent inhibition while the light was on (figure 36 H and I). The intensity dependent decrease in the interflash spike frequency as measured 30 seconds after a light flash (in Ringer)suggests that effective concentrations of receptor transmitter remain long after the end of the stimulus (figure 30). However, the observation that a low intensity stimulus can cause a more severe decrease in interflash spike frequency in the presence of a low dose of curare than it would in Ringer is not consistent with this idea (figure 29).

Although the notion that the spiking neuron is receiving excitatory GABAnergic input from adjacent ocellar nerve dendrites and inhibitory cholinergic input from

photoreceptors is intriguing and consistent with most of the experimental findings, an alternate possibility could be that the spiking neuron is third or higher order and is receiving input from a second order neuron.

## Pharmacology

### Effect of Curare on the light response and interflash frequency

Curare is a classical nicotinic cholinergic antagonist which acts by competing for the acetylcholine binding site on the post-synaptic membrane (Koelle, 1975b). Its effect on the light-induced inhibition of impulse activity (figures 22 and 27) is consistent with evidence obtained by other investigations which suggests that acetylcholine is the transmitter, which is released in increasing amounts in response to light, and causes a graded hyperpolarization of the second order cell (Dowling and Chappell, 1972; Klingman and Chappell, 1978; Stone, 1982).

It has already been shown that curare can alter the light evoked "off" response observed in the median ocellar photoreceptors (Stone and Chappell, 1981) and block the L-neuron's response to light (Klingman and Chappell, 1978). Not only has this investigation shown that curare can also affect the ocellar nerve impulse activity, but it has revealed some interesting effects of low concentrations of the drug on ocellar physiology that were not clearly

discernible using intracellular recording techniques (Stone, 1982).

At low doses, curare decreased the slope of the intensity vs frequency (impulse) relationship (figures 31 and 32). This effect can be seen after the addition of as little as 50 um of curare to the bathing medium. Further increases in drug dose shifted this relationship to the right, without a further change in slope. Another way of expressing this finding is to say that during illumination, 50 uM of the drug decreased the impulse frequency when compared to the control (Ringer) values (figure 22B), while higher curare concentrations effected a dose-dependent increase in the impulse activity from that level. The former was an unexpected finding.

Classically speaking, the greater the degree of presynaptic depolarization the greater the release of transmitter (Katz and Miledi, 1965; Katz and Miledi, 1967). Since there is a direct relationship between the light intensity and the level of depolarization recorded in the receptor terminals (Chappell and Dowling, 1972; Stone, 1982), it is assumed that a graded increase in light intensity should result in graded amounts of transmitter release. Therefore figure 31 may be thought of as a series of dose-response curves. That is, the relative

dose of receptor transmitter that causes a given reponse in the presence and absence of a competitive inhibitor (curare).

Competitive inhibition is conventionally displayed as a parallel shift to the right of the semi-log plot of a dose-response relationship (Jenkinson, 1960; Hollenberg, 1978). In other words, in the presence of curare, more transmitter (a higher intensity light) should be needed to evoke the same magnitude of response seen in the absence of the drug, without decreasing the maximum response. The finding that a low dose of curare changed the slope of the intensity- frequency relationship suggests that curare may not be acting competitively on the spiking neuron. This could be explained by postulating that either ocellar nerve impulse frequency is not solely modulated by the receptor transmitter, or that there is auto-inhibition of receptor transmitter release. Both of these possibilities are compatible with the dual threshold model.

Auto-inhibition or lateral inhibition of photoreceptors is supported by a number of observations. At low doses (50 uM), curare appeared to lower the interflash spike rate when measured 30 seconds after a dim or intermediate intensity light stimulus. However, it did not af-

fect the spike rate recorded from a dark adapted eye (figure 29). A similar effect has been seen during intracellular recordings from median ocellar L-neurons. Some of these cells exhibited a small membrane hyperpolarization for several seconds after light "off" in the presence of this drug (Stone, personal communication).

Fifty micromolar curare decreased the gain of the spiking neuron's response to light (observe the change in slope in figure 34), but lowered the response threshold at low light intensities (figure 33). Intracellular recordings from median ocellar L-neurons which were perfused with curare also showed an initial decrease in the response threshold when the drug was first applied. This was eventually followed by a reduction or loss of the light response (Stone, 1982).

Anatomical studies have shown that the dyadic synapses from photoreceptor terminals make synaptic connections onto adjacent photoreceptor terminals, as well as ocellar nerve dendrites. This synaptic anatomy strongly suggest that there are lateral interactions between receptor cells (Stone, 1982).

If these lateral interactions were inhibitory then a dose specific selective action by curare on these terminal binding sites could result in a reduction

of the lateral inhibitory effects. This would lead to an increased release of acetylcholine onto the ocellar nerve dendrites during the interflash period causing 1. the spike firing rate in the spiking neuron to decrease; and 2. the membrane potential of the L-neuron to hyperpolarize for a few seconds after light "off".

At light "on", a blockage of lateral inhibitory interactions by a low dose of curare could result in a larger amount of receptor transmitter being released due to light. This would result in a larger response to illumination in the second or higher order cells and would account for the apparent increased sensitivity seen in intracellular recordings from L-neurons and extracellular spike analysis from spiking neurons.

However, the decrease in gain seen after the addition of 50 uM curare cannot be explained solely on the basis of an inhibition of lateral interactions between receptor cells since a decrease in lateral inhibition should result in an increase in gain. It may be that another transmitter is needed to modulate ocellar sensitivity. Perhaps the release or the action of this other transmitter is altered in the presence of curare resulting in the leveling of the slope for these curves (figure 32 and 34).

Effects of Curare on the dark firing rate

Besides its effect on the light response, curare caused an increase in the impulse firing rate in the dark which was dose dependent over a certain range of concentrations (figures 22A and 29). This would be expected if the photoreceptors spontaneously release some transmitter in the dark (Klingman and Chappell, 1978). Since spike frequency is proportional to membrane potential (Katz, 1950), if the increase in dark and interflash impulse frequency were due to curare's action as a cholinergic antagonist at the post-synaptic membrane of the dendrites, than one would expect to see a significant drug induced depolarizing change in the L-neuron dark resting potential in the presence of this drug. Such a change was in fact reported and is consistent with the present findings (Stone, 1982).

This supports the idea that spike frequency is a reliable probe of changes in membrane potential. Whereas changes in potential of only a few millivolts are often hard to quantify because of the perturbations of the baseline as seen on an oscilloscope, small changes in membrane potential may be quite significant in terms of modulating spike frequency. Also since extracellular recordings are non-invasive, uncertainty as to the effect of cell damage

on intracellular voltage measurements are eliminated. For these reasons, extracellular spike frequency measurements appears to be a useful way to monitor minute changes in intracellular potential.

Lateral inhibitory interactions between photoreceptors

The selectivity of drug action is two-fold, first it is dose dependent in that a low dose decreases the response threshold whereas higher doses raise this threshold (figure 33). Second, its effect on the inter-flash spike frequency occurs only after a light flash. If curare merely competes with acetylcholine for nicotinic binding sites, then transmitter concentrations within the synaptic cleft should affect its actions. It is unlikely that curare is affecting GABA receptors in the ocellus (if they exist) but this is a possibility. However, in one study on the effects of GABA in Bullfrog sympathetic ganglion, curare was routinely added to the bathing medium of an in vitro preparation. No effect of curare was reported after the application of the test dose of GABA (Kato, et. al., 1980).

One reasonable explanation for the selectivity of curare is as follows. Assuming there is a resting release

of acetylcholine (see Background Information: Synaptic Feedback Model), the amount of photoreceptor transmitter released in the light may be orders of magnitude greater then the spontaneous dark release, so that in the light, at low drug concentrations, the curare/acetylcholine ratio in the synaptic cleft might be too small to have a large effect on the light response. But right after a bright flash the amount of acetylcholine would decrease to some concentration greater than in the dark adapted state though less than what it is during illumination. At this time a low dose of curare could successfully compete with the transmitter resulting in its effect on the interflash spike frequency. After an hour of dark adaptation, the spontaneous release of receptor transmitter would have dropped so as to preclude action by any concentration of curare (no transmitter so nothing to block). This would explain why 50 um curare affected the interflash spike frequency without affecting the dark adapted impulse rate.

If there is no acetylcholine to interact with in the dark, why does the dark adapted spike frequency become dose dependent to curare concentrations over 100 uM (figure 29)? Why does 50 uM curare decrease the threshold at which the impulse activity is affected by light? In addi-

tion, if curare blocks cholinergic post-synaptic binding sites why does this low dose cause a decrease instead of an increase in frequency?

One way to get around this dilemma is to postulate that there are presynaptic cholinergic binding sites on the photoreceptors and that these binding sites have a higher affinity for curare than the cholinergic sites of the dendrites of the spiking cell. In this way the dose dependency would be due to the difference in binding constants between the two classes of receptors (Koelle, 1961). That is a low dose would have a minimal effect on synaptic transmission between photoreceptors and second order cells, while having a larger effect on the suggested lateral inhibitory interactions between neighboring photoreceptors. For example, blocking the lateral inhibitory interactions of the photoreceptors at low light intensities would cause a greater release of receptor transmitter and result in an apparent decrease in response threshold. The change in gain could be accounted for by assuming that curare has some effect on the synapse between the first and second order cells. The effect of the drug on the interflash spike frequency could be due to an interruption of the system's reset mechanism. Perhaps these postulated lateral synapses work to reduce the

amount of tranmitter released under all circumstances. By interferring with this process more acetylcholine is released after a light stimulus than normally would be, and so it takes the system longer to return to its dark steady state.

One other mechanism might account for the effect of curare on the interflash spike frequency. Since a low dose seems to affect the spike frequency only after a light flash, it seems resonable to suggest that curare may be affecting the enzyme acetylcholinesterase, which is believed to degrade the receptor transmitter (Koelle, 1975a). One would imagine that the greater the intensity of light falling on the ocellus, the greater the amount of acetylcholine will be released within the synaptic plexus, and the longer it would take the esterase to reduce the concentration of transmitter within the synaptic cleft. This would explain the intensity dependent decrease in interflash spike frequency in the absence of any drug. If cholinesterase activity were decreased by curare, it would take longer to lower the acetylcholine concentration in the subsynaptic space, which could explain the drug-induced membrane hyperpolarization observed in the L-neuron seconds after light "off" (Stone, personal communication) as well as the drug-induced decrease

in interflash spike frequency. After an hour of dark adaptation, as long as the curare concentration was low, the esterase should be able to lower the concentration of acetylcholine sufficiently so as to result in a normal impulse rate. Although this explanation agrees with some of the experimental observations, it does not take into account the dose-dependent increase of interflash spike frequency at higher drug doses. If curare's action were on the cholinesterase molecule, the synaptic concentration of acetylcholine would increase with increased dosage because of the impediment to inactivation. Ultimately this would lead to a dose-dependent decrease of spike frequency in the dark, and in fact the opposite is seen.

It therefore appears more probable that the effect of curare in low doses on the dark firing rate may be mediated by a reduction in the lateral inhibitory interactions postulated to exist between neighboring photoreceptors. The effect of higher doses of this drug may be due to it's action on the post-synaptic cholinergic binding sites presumed to exist on the dendrites of the L-neuron. The results suggest that the proposed presynaptic cholinergic binding sites which may mediate lateral interactions between photoreceptors are differentially

more sensitive to curare than those thought to exist on the post-synaptic membranes of the L-neuron.

Effect of GABA on spontaneous impulse activity in the dark

The GABA-induced inhibition of spontaneous ocellar nerve impulse activity (figures 35 and 36) is consistent with the evidence from other studies that there is a GABAnergic component in the dragonfly's ocellar system (Klingman and Chappell, 1978; Stone and Chappell, 1981; Stone, 1982). It has been proposed that GABA functions as a feedback transmitter, released by median ocellar L-neurons back onto photoreceptors terminals where it facilitates the release of receptor transmitter (Klingman and Chappell, 1978; Stone and Chappell, 1981). Since anatomical studies have revealed what appear to be lateral synaptic connections between dendrites of the L-neurons (Dowling and Chappell, 1972), it seems appropriate to propose that GABA also serves as the transmitter which mediates these presumed lateral interactions between adjacent ocellar nerve dendrites (Stone, 1982).

If one assumes that the drug-induced changes in the behavior of the spiking activity were synaptically mediated, then GABA could either be working directly by binding

to GABA receptors on the membrane of the spiking neuron, indirectly by modulating release of receptor transmitter, or by some combination of these two mechanisms.

There are three possibilities by which GABA could work directly at a synaptic site to inhibit spontaneous impulse activity. GABA could hyperpolarize the membrane below the threshold for firing an impulse, depolarize the neuron enough to result in a depolarization block at the site of spike initiation, or clamp the neuron below its spike firing threshold. The last mechanism differs from the previous two in that it suggests that a conductance channel can be opened for an ion or ions whose reversal potential is between the two thresholds (see: Discussion of dual threshold model). If the conductance is sufficiently large, it could prevent spiking activity by acting as a shunt for electrotonic currents. This possibility has not been tested in this preparation. There is much evidence that a GABAergic neuron effects presynaptic inhibition in the ventral horn of the mammalian spinal cord by depolarizing presynaptic terminals (Levy, 1977). A similar action has been observed in the lateral olfactory tract of rat where the GABA induced depolarization is dose related (Pickles, 1979).

A direct action assumes that the overwhelming effect af GABA is accomplished through lateral synaptic interactions between ocellar nerve dendrites (L-neurons or spiking neurons), and this is by no means clearly proven. A simpler explanation might be that the putative transmitter, GABA, is facilitating the release of the receptor transmitter, and since the experiments using curare suggest that the acetylcholine reduces impulse activity (Chappell and Dowling, 1972), GABA could be acting indirectly by causing a massive release of receptor transmitter resulting in an inhibition of spikes.

Effect of cobalt on impulse activity

The preparation was perfused with 12 mM cobalt so that spike frequency could be measured in the absence of synaptic transmission. Cobalt is known to block release of chemical transmitters by competing with calcium (Weakly, 1973). Reports have been published that it blocks the release of acetylcholine at the frog neuromuscular junction (Weakly, 1973) and serotonin and norepinephrine in rat and cat spinal cord (Yaksh and Tyce, 1980). In invertebrate preparations it is thought to block synaptic

transmission at concentrations of up to 40 mM (Zimmerman, 1978; Alkon and Grossman, 1978). However, it has been shown to block the intracellularly recorded light response in median ocellar L-neurons as well as altering the "off" response in dragonfly ocellar photoreceptors at concentrations as low as 6 mM (Stone and Chappell, 1981; Stone, 1982).

There was an increase in the interflash spike frequency after perfusing the preparation with this ion for five minutes, and the impulse rate in the light was no longer completely inhibited (figure 39). However, by 18 minutes, all impulse activity had dropped to zero. Assuming that cobalt was not acting to block impulse generation at the initiation site, then the results suggest that some synaptic input is needed to facilitate the appearance of the spiking activity. Since there is strong evidence that the presumed receptor transmitter inhibits impulse activity, it seems likely that the transmitter released from the ocellar nerve dendrites is responsible for facilitating impulse activity. These results are consistent both with the synaptic feedback model (Klingman, 1976; Stone, 1982) and the dual threshold model discussed earlier in this paper.

GABAs effect on the light response

One interesting finding is that GABA did more than eliminate spontaneous impulse activity in the dark. In the presence of 10 mM GABA, light flashes at intensity levels between log I=-6 and log I=-3 facilitated the appearance of ocellar nerve action potentials (figures 36 and 37). Greater intensities resulted in a restoration of the interflash impulse activity and its inhibition by light. According to the dual threshold model, the reappearance of the interflash spiking activity above the stimulus intensity of log I=-2 results from a tremendous GABA induced enhancment of the "off" response (figure 36). The model assumes that the light intensities at which interflash spiking is seen are so bright that the period between consecutive stimuli is not long enough to allow the impulse rate to fall to zero.

It is conceivable that GABA works to inhibit the spiking neuron in the dark by causing release of receptor transmitter. However, it should be noted that the first effect of GABA is to prevent the complete light mediated inhibition of impulse activity (figure 35). Also, before the dark frequency was blocked an

increase in interflash impulse rate occurred which lasted approximately 7 minutes. From these results it appears that GABA does not directly inhibit the spiking neuron by effecting a membrane hyperpolarization. If it did, it is unlikely that any impulse activity would appear in response to light and that interflash frequency would rise before it fell during the time of drug perfusion.

It has been postulated that the dendrites of the spiking neuron take part in reciprocal and lateral synaptic interactions. If it is assumed that these lateral interactions are inhibitory, it would mean that the GABA released in the dark, although facilitating receptor transmitter release, would limit its own release. If this were so, then it would be difficult to explain the spiking activity seen during light stimulation, since receptor transmitter is assumed to be inhibitory. It does not seem logical that two inhibitory transmitters acting at the same time would result in a depolarizing action.

This observation raises the possibility that there may be a GABAnergic component within the dragonfly ocellar system which appears to help in the modulation of the spiking activity in a facilitory fashion. The mechanism

of GABA's action may be via a depolarizing block, although other possibilities cannot be ruled out.

# CONCLUSIONS

A means of recording ocellar nerve impulse activity extracellularly from a single ocellar nerve has been developed. The results have made it apparent that the corneally recorded impulses are originating from the lateral ocellar nerves and not the median ocellar nerve, the antennal nerve, optic lobes, or ventral nerve cord. These spikes appear to be sodium dependent and have a lower response threshold to green light (485 nm) than they do for ultraviolet light (360 nm).

Evidence has been provided which strongly suggests that the slow potential responses of the L-neuron may be the normal response of this neuron and that it is not a damaged spiking cell. Also, the obvious latency between ocellar nerve action potentials in a single lateral ocellar nerve is in accord with the idea that there is only one spiking cell which produces large light inhibited spikes in each lateral ocellar nerve.
It could not be determined whether the cell which is generating the corneally recorded impulses is a second or higher order neuron, but the results suggest that the pattern of the impulse activity is what one would expect if the impulse generating cell were receiving synap-

tic input from the photoreceptor. This is consistent with previous investigations which have reported that the large light inhibited spikes seen extracellularly are afferent in nature (Rosser, 1974; Kondo, 1978). Although this thesis has not addressed itself to the role of the ocellar impulse activity in dragonfly behavior, future studies will be able to use the information that it has provided to support or eliminate competing possibilities.

A preparation has evolved, which can be used to study the effects of pharmacological agents within the ocellus for periods of up to 12 hours. In addition, a means of collecting and analyzing impulse frequency data by computer was developed. This thesis has revealed a biphasic effect of curare on impulse frequency during illumination which is dose related, and a biphasic effect of this drug on the interflash spike frequency. Also, low doses of curare appear to lower the stimulus threshold at which impulse activity responds to light. This is particularly fascinating since a similar effect of a low dose of this drug was reported to occur in the L-neuron (Stone, 1982). It seems that the the most likely explanation for curare's ability (at lower doses) to decrease the response threshold of the

impulse activity and to lower the interflash spike frequency, may be due to a dose selective effect on the presumptive lateral interactions between photoreceptors. However, further research is needed to determine if this is indeed the mechanism by which curare mediates these effects.

GABA has been shown to inhibit spontaneous impulse activity in the dark, and cause a light dependent increase in spike frequency which was shifted towards light "off" at high light intensities. Cobalt blocked all impulse activity at a concentration of 12 mM. A model was proposed to explain these results suggesting that the spiking neuron has an upper and lower threshold for impulse initiation. One can conclude that GABA does affect ocellar impulse activity but it is unclear by what means it accomplishes its action. One possibility is that it works by depolarizing the dendrites of the spiking cell. This explanation is consistent with much of the data, but it does not provide a mechanism by which a high firing rate abruptly decreases to zero at the site of spike initiation as the membrane potential crosses the upper threshold. Clearly further investigations are needed to clarify this issue.

Since all of the agents shown to affect spiking

activity have been reported to effect the L-neuron, there are two interesting questions that have yet to be answered. 1. Is the neuron generating the impulse activity studied in this thesis, a third or higher order cell receiving synaptic information from the L-neuron, or is it a second order cell? and 2. Is spiking activity originating from a spiking L-neuron or is it coming from a fiber with a smaller diameter (s-neuron) within the ocellar nerve? Clearly intracellular recordings from the spiking cell followed by iontophoretic staining would be very helpful in answering these questions.

In conclusion, the stability of the extracellular dragonfly preparation makes it particularly amenable to pharmacological studies. The questions that have resulted from this thesis have opened up exciting possibilities for future research which include: determining if GABA acts directly on the spiking cell or if it acts indirectly by facilitating receptor transmitter release; use of other cholinergic agents other than curare to selectively affect the postulated presynaptic cholinergic binding sites on the photoreceptors; and quantifying curare's effect on the time course of the return of spike frequency to it's dark adapted rate. Also the preparation might be useful as a physiological assay for studying the relative potency of

cholinergic and GABAnergic agents.

The relative ease with which one can make intracellular recordings from the neurons of the dragonfly ocellus make it ideal for correlating the extracellular pharmacological data generated by this thesis with the cellular physiology of this preparation. In the future, correlations of this type should help in elucidating the means by which simple neuronal systems integrate sensory information.

## SUMMARY

1. The large light-inhibited action potentials which can be recorded from the cornea of any of the three ocelli appear to be originating in the lateral ocellar nerves. Ablation of one lateral ocellar nerve results in a loss of half of the spikes. Ablation of both lateral ocellar nerves eliminated all the spikes.

2. Ablation of the median ocellar nerve has no effect on corneally recorded light-inhibited impulse activity, suggesting that it is not normally present in the median ocellar nerve.

3. The interspike interval pattern of ocellar nerve impulses in a single lateral ocellar nerve suggests that there is only one spiking neuron in each lateral ocellar nerve which produces the corneally recorded large light-inhibited action potentials.

4. The first intracellular recordings from the dragonfly lateral ocellar nerve showed a spiking neuron (resting potential = -55 mv) with light inhibited action potentials 30 mv in amplitude. Non-spiking neurons were also

recorded from. These cells had resting potentials ranging from -30 mv to - 55 mv. Cells showed a hyperpolarizing response to light identical to that recorded from L-neurons in the median ocellus.

5. Simultaneous intracellular recording from a non-spiking neuron and extracellular recording of spiking activity revealed that typical L-neuron responses can be recorded without eliminating the impulse activity suggesting that L-neuron slow potential responses may be the normal response for these cells and not a damaged spiking cell.

6. Intracellular recording and subsequent staining with cobalt shows a non-spiking neuron with a dendritic field in the lateral ocellar cup sending an ipsilateral process into the brain.

7. 0.3 uM TTX eliminated all spiking activity, suggesting that this activity is sodium dependent.

8. The extracellularly recorded spikes were more sensitive to green light (485 nm) than to UV light (360 nm) and, unlike the non-spiking ocellar neurons, there was no

reverse "Purkinje shift".

9. The impulse activity was shown to be perturbed by agents which affect the L-neuron.

10. A means of collecting and analyzing impulse frequency data by computer was developed and applied to analyzing the effects of these agents.

11. 12 mM cobalt inhibited spontaneous impulse activity.

12. After 56 minutes 10 mM GABA inhibited spontaneous impulse activity in the dark and caused a light dependent increase in spike frequency which was shifted towards light "off" at high light intensities.

13. A model was proposed to explain these results suggesting that the spiking neuron has an upper and lower threshold for impulse initiation.

14. Curare at concentrations of 100 uM or greater causes increasing block of the inhibition of dragonfly lateral ocellar nerve impulses, as dose increases.

15. Curare prolongs the time course of the return to the dark frequency of impulse activity.

16. Effect #14 is dominant at higher doses (100 uM curare and above) while effect #15 is dominant at lower doses (50 uM curare). It is not clear whether the prolongation of the return of dark frequency and block of inhibition involve the same, separate or interrelated mechanisms.

17. The low dose (50 uM curare) results in an increase in the steady state (plateau phase) sensitivity evidenced by a lower threshold for changes in impulse firing rate measured during the plateau phase (last 1.5 seconds) of a two second flash.

18. The low dose (50 uM curare) leaves the saturating intensity (log I=-5) unchanged, resulting in a change in slope of the intensity-response curves. This may represent a reduced gain in the response of the system to light. This slope remains the same at all drug doses tested (50 uM to 1000 uM), even when saturation occurs before complete block of impulse activity (500 uM and 1000 uM).

19. A similar change in slope is noted in the intensity response curves obtained for the change in impulse firing rate between the steady state (plateau phase) light frequency and the interflash dark frequency.

20. The effects of low doses of curare on the interflash spike frequency may be due to its effect on the presumptive lateral interactions between adjacent photoreceptor terminals.

# Appendix

## Spikes

This is a Basic program designed to run on a Digital Equipment PDP-11 MINC computer. It is capable of counting nerve impulses per interval time and storing these counts in a virtual array.

```
20 PRINT 'If you havent already done so be sure to use
   the EXTRA_SPACE ';
21 PRINT 'command.'
22 PRINT  \ PRINT  \ PRINT  \ PRINT  \ PRINT
26 PRINT 'There are 60 intervals per run and three runs
   per light intensity.';
30 PRINT 'The minimum allowed time (due to sampling speed)
   per interval is ';
35 PRINT ' 0.2 seconds....there is no maximum allowed
   time.'
92 SET_LINE(0,1,0)
93 SET_LINE(2,1,0)
94 PRINT  \ PRINT
```

```
101 PRINT "how many seconds per interval"; \ INPUT R
111 D=R*100
121 DIM A(36,60)
131 PRINT "At the end of what interval # will the light
    go   ON  "; \ INPUT F
161 PRINT "at the end of what interval # will the light
    go   OFF  "; \ INPUT H
171 PRINT 'How many drug doses' \ INPUT L
172 PRINT  \ PRINT
181 FOR P=1 TO L
185 IF L=1 GO TO 211
187 IF L=2 GO TO 202
189 IF L=3 GO TO 203
191 IF L=4 GO TO 204
193 IF L=5 GO TO 205
195 IF L=6 GO TO 206
197 IF L=7 GO TO 207
202 ON P GO TO 211,281
203 ON P GO TO 211,281,281
204 ON P GO TO 211,281,281,281
205 ON P GO TO 211,281,281,281,281
206 ON P GO TO 211,281,281,281,281,281
207 ON P GO TO 211,281,281,281,281,281,281
211 PRINT 'Enter the drug at its concentration....eg.
```

```
    curare 10-3 M = cur3'
241 PRINT
271 PRINT  \ PRINT '       eg.     sys:filename';
    \ INPUT A$
272 GO TO 360
281 PRINT 'Do you want to continue (type y or n )';
    \ INPUT X$
282 IF X$='n' GO TO 19998
283 PRINT 'What is the next drug concentration:'
284 IF P=2 GO TO 311
287 IF P=3 GO TO 314
290 IF P=4 GO TO 317
293 IF P=5 GO TO 320
296 IF P=6 GO TO 323
311 INPUT B$ \ GO TO 360
314 INPUT C$ \ GO TO 360
317 INPUT D$ \ GO TO 360
320 INPUT E$ \ GO TO 360
323 INPUT F$ \ GO TO 360
360 PRINT 'How many different intensities per drug dose';
    \ INPUT W
362 PRINT  \ PRINT
365 I=0 \ J=0
370 FOR N=1 TO W
```

```
375 T=3*N
380 PRINT 'Do you want to continue  (type y or n )';
    \ INPUT X$
385 IF X$='n' THEN T=T-3 \ IF X$='n' GO TO 17020
386 PRINT  \ PRINT
390 PRINT 'Enter the NO.('; \ PRINT N; \ PRINT ') light
    intensity as a ';
391 PRINT 'negative number.    eg. (-4   represents
    Ln I=-4)'
392 PRINT  \ PRINT
395 PRINT ; \ PRINT 'Ln I  ='; \ INPUT S
400 PRINT  \ PRINT  \ PRINT  \
500 V=-1
505 B=0 \ A=0
515 Z=0
520 SCHMITT(2,540) \ SCHMITT(1,10150,D)
525 GO TO 526
526 IF Y=1 GO TO 17000
535 GO TO 525
540 A=B+1
545 B=A
550 RETURN
800 ON P GO TO 1000,1200,1400,1600,1800,2000,2200
1000 OPEN A$ AS FILE #1
```

```
1010 DIM #1,B(36,60)
1020 FOR J=1 TO T
1030 K=K+1
1040 B(J,0)=A(J,0) \ PRINT 'Ln I  ='; \ PRINT B(J,0)
1050 FOR I=1 TO 60
1060 B(J,I)=A(J,I)
1070 PRINT B(J,I);
1080 NEXT I
1090 PRINT  \ PRINT
1100 IF K=3 THEN PRINT  \ IF K=3 THEN PRINT
1110 IF K=3 THEN K=0
1120 NEXT J
1130 PRINT  \ PRINT
1140 CLOSE #1
1150 GO TO 10080
1200 OPEN B$ AS FILE #2
1210 DIM #2,C(36,60)
1220 FOR J=1 TO T
1230 K=K+1
1240 C(J,O)=A(J,0) \ PRINT 'Ln I  = '; \ PRINT
     C(J,0)
1250 FOR I=1 TO 60
1260 C(J,I)=A(J,I)
1270 PRINT C(J,I);
```

```
1280 NEXT I
1290 PRINT  \ PRINT
1300 IF K=3 THEN PRINT  \ IF K=3 THEN PRINT
1310 IF K=3 THEN K=0
1320 NEXT J
1330 PRINT  \ PRINT
1340 CLOSE #2
1350 GO TO 10080
1400 OPEN C$ AS FILE #3
1410 DIM #3,D(36,60)
1420 FOR J=1 TO T
1430 K=K+1
1440 D(J,0)=A(J,0) \ PRINT 'Ln I  = ';
     \ PRINT D(J,0)
1450 FOR I=1 TO 60
1460 D(J,I)=A(J,I)
1470 PRINT D(J,I);
1480 NEXT I
1490 PRINT  \ PRINT
1500 IF K=3 THEN PRINT  \ IF K=3 THEN PRINT
1510 IF K=3 THEN K=0
1520 NEXT J
1530 PRINT  \ PRINT
1540 CLOSE #3
```

```
1550 GO TO 10080
1600 OPEN D$ AS FILE #4
1610 DIM #4,E(36,60)
1620 FOR J=1 TO T
1630 K=K+1
1640 E(J,0)=A(J,0) \ PRINT 'Ln I  = '; \ PRINT E(J,0)
1650 FOR I=1 TO 60
1660 E(J,I)=A(J,I)
1670 PRINT E(J,I);
1680 NEXT I
1690 PRINT  \ PRINT
1700 IF K=3 THEN PRINT  \ IF K=3 THEN PRINT
1710 IF K=3 THEN K=0
1720 NEXT J
1730 PRINT  \ PRINT
1740 CLOSE #4
1750 GO TO 10080
1800 OPEN E$ AS FILE #5
1810 DIM #5,F(36,60)
1820 FOR J=1 TO T
1830 K=K+1
1840 F(J,0)=A(J,0) \ PRINT 'Ln I  = '; \ PRINT F(J,0)
1850 FOR I=1 TO 60
1860 F(J,I)=A(J,I)
```

```
1870 PRINT F(J,I);
1880 NEXT I
1890 PRINT  \ PRINT
1900 IF K=3 THEN PRINT  \ IF K=3 THEN PRINT
1910 IF K=3 THEN K=0
1920 NEXT J
1930 PRINT  \ PRINT
1940 CLOSE #5
1950 GO TO 10080
2000 OPEN F$ AS FILE #6
2010 DIM #6,G(36,60)
2020 FOR J=1 TO T
2030 K=K+1
2040 G(J,0)=A(J,0) \ PRINT 'Ln I  = '; \ PRINT G(J,0)
2050 FOR I=1 TO 60
2060 G(J,I)=A(J,I)
2070 PRINT G(J,I);
2080 NEXT I
2090 PRINT  \ PRINT
2100 IF K=3 THEN PRINT  \ IF K=3 THEN PRINT
2110 IF K=3 THEN K=0
2120 NEXT J
2130 PRINT  \ PRINT
2140 CLOSE #6
```

```
2150 GO TO 10080
10080 PRINT 'File #'; \ PRINT P; \ PRINT ' has been
      opened and the data has ';
10090 PRINT 'been transferred.  This file is now
      CLOSED !'
10100 PRINT  \ PRINT
10125 GO TO 18000
10150 IF V=-1 GO TO 10320
10170 IF J=0 THEN G=1
10180 J=G
10181 A(J,0)=S
10190 I=I+1
10200 IF I>60 THEN J=G+1
10210 G=J
10220 IF I>60 THEN I=0
10225 IF J=37 THEN J=0
10230 A(J,I)=B
10240 B=0
10260 U=V+1
10270 V=U
10280 IF V=F GO TO 10380
10290 IF V=H GO TO 10410
10300 IF V=61 THEN V=-1
10310 RETURN
```

```
10320 IF Z=3 GO TO 12000
10330 Z=Z+1
10340 B=0
10350 SET_LINE(0,0,0)
10360 SET_LINE(0,1,0)
10370 GO TO 10260
10380 SET_LINE(2,0,0)
10390 B=0
10400 RETURN
10410 SET_LINE(2,1,0)
10420 B=0
10430 RETURN
12000 Y=1
12010 SCHMITT \ SCHMITT(2)
12020 RETURN
17000 Y=0
17010 NEXT N
17020 GO TO 800
18000 NEXT P
19998 PRINT  \ PRINT  \ PRINT  \ PRINT  \ PRINT
      \ PRINT  \ PRINT  \ PRINT
19999 PRINT ; \ PRINT ; \ PRINT '   THE EXPERIMENT
      IS OVER  '
20000 END
```

Multispikes

This program will count impulses per interval time for a series of stimuli (see "Methods:Experimental protocol for pharmacological experiments). The counts are stored in a virtual array and a bargraph of the data is then automatically constructed.

```
1 SET_LINE(2,0) \ SET_LINE(0,1)
3 PRINT "type in the dose and name of drug... eg.
  4 x E05 M  CUR"; \ INPUT B$
4 PRINT 'What is the file name '; \ INPUT A$
5 PRINT "How many seconds per interval "; \ INPUT A
  \ B=100*A
10 PRINT 'At the end of what interval will the light go
   ON  '; \ INPUT D
20 PRINT "At the end of what interval will the light go
   'OFF' "; \ INPUT E
22 PRINT "At the end of what interval will the counting
   stop "; \ INPUT K
25 PRINT "At the end of what interval will the cycle
   repeat "; \ INPUT F
27 PRINT "How many cycles per light intensity ";
```

```
   \ INPUT H
28 PRINT "How many light intensities per drug ";
   \ INPUT L
30 DIM A(6,100)
35 DIM Y(6,100)
37 L=L+1
40 FOR J=1 TO L
41 V=-1
42 IF J=L GO TO 7000
43 I=0
44 SET_LINE(0,1)
45 IF J>1 GO TO 55
50 PRINT "What is the light intensity "; \ INPUT C
51 GO TO 60
55 PRINT "What is the number _"; \ PRINT J;
   \ PRINT "_ light intensity "; \ INPUT C
60 A(J,0)=C
95 SCHMITT \ SCHMITT(2)
100 SCHMITT(1,500,B) \ SCHMITT(2,110)
101 GO TO 106
106 IF G=H GO TO 5000
109 GO TO 101
110 N=N+1 \ RETURN
500 IF V=-1 THEN SET_LINE(0,0,0)
```

```
501 SET_LINE(0,1,0)
502 IF V=-1 THEN N=0
510 V=V+1
520 IF V=0 THEN RETURN
530 I=I+1
535 IF I>K GO TO 550
540 A(J,I)=A(J,I)+N
550 N=0
560 IF V=D THEN SET_LINE(2,1)
570 IF V=E THEN SET_LINE(2,0)
575 N=0
580 IF V=F THEN I=0
581 IF V=F THEN G=G+1
582 IF V=F THEN V=-1
585 IF G=H THEN SCHMITT \ IF G=H THEN SCHMITT(2)
587 IF G=H THEN I=0
590 RETURN
600 NEXT J
5000 G=0
5055 M=K-1
5057 PRINT J
5060 FOR I=1 TO M
5070 Y(J,I)=A(J,I)
5074 NEXT I
```

```
5075 DISPLAY_CLEAR
5076 PRINT "          Intensity    LN  I    = ";
     \ PRINT A(J,0)
5080 WINDOW(,,,,300)
5081 BARGRAPH("-ticks",100,,Y(J,1),,,1)
5082 LABEL(,B$,"no of AP s")
5090 GO TO 600
7000 OPEN A$ AS FILE #1
7010 DIM #1,B(6,100)
7015 DISPLAY_CLEAR
7020 FOR J=1 TO 6
7030 B(J,0)=A(J,0) \ PRINT B(J,0)
7040 FOR I=1 TO 100
7050 B(J,I)=A(J,I)
7060 PRINT B(J,I);
7070 NEXT I
7080 PRINT
7090 NEXT J
7100 CLOSE
7200 WINDOW(,,,,300)
7210 BARGRAPH(,,,Y(1,1))
7300 LABEL(,B$,"No of AP s")
10000 END
```

# REFERENCES

Adrian, E.D. and Zotterman, Y. (1926). The impulses produced by sensory nerve endings. II. The response of a single end-organ. J. Physiol. (Lond.) 61:151-171.

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

Armstrong, C.M. and Benzanilla, F. (1974). The charge movement associated with the opening and closing of the activation gates of the Na channels. J. of Gen. Physiol. 63:533-552.

Ashmore, J.F. and Copenhagen, D.R. (1980). Different post synaptic events in two types of retinal bipolar cell. Nature 288:84-86.

Bacon, J.P and Altman, J.S. (1976). A silver intensification method for cobalt-filled neurones in whole-mount preparations. Brain Res. 138:359-363.

Barlow, R.B.; Chamberlain, S.C.; Kaplan, E. (1977). Efferent inputs and serotonin enhance the sensitivity of the Limulus lateral eye. The Biol. Bull. 153: 414.

Bortoff, A. (1964). Localization of slow potential responses in the Necturus retina. Vision Research 4: 626.

Cajal, S.R. (1894). Die retina der wirbethiere. Translated by Greef, Wiesbaden, 1894, Bergman.

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

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

Calvin, W.H. and Graubard, K. (1979). Styles of neuronal computation. In: The Neurosciences, Fourth study program, Schmitt, F.O. and Worden, F.G. (Eds.). Cambridge, Mass: MIT Press, pp 513-524 .

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

Chappell, R.L. and DeVoe, R.D. (1975). Action spectra and chromatic mechanisms of cells in the median ocelli of dragonflies. J. Gen. Physiol. 60:399-419.

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

Chappell, R.L. and Kuhar, M.J. (1976). Evidence for Ach in dragonfly ocellar retina. Association for Research in Vision and Opthalmology, Abstract: Spring meeting Vol. 17.

Cornwall, P.B. (1955). The function of the ocelli of Calliphora and Locusta. J. of Exp. Biol. 32.

Defeudis, F.V. (1977). Receptors in the vertebrate nervous system. Prog. in Neurobiol. 9:123-145.

Dethier, V.G. (1942). The dioptric apparatus of lateral ocelli. J. of Cellular and Comp. Physiol. 19:301-313.

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

Dowling, J.E. and Boycott, B.B. (1966). Organization of the primate retina: electron microscopy. Proc. Roy. Soc. B. 166:80.

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

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

Duchateau, G.; Florkin, M.; 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.

Freeman, A.R. (1973). Electrophysiological analysis of the actions of strychnine, bicuculline, and picrotoxin on the axonal membrane. J. Neurobiol. 4:565-582.

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

Gerschenfeld, H. M. and Piccolino, M. (1979). Pharamcology 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 213-226.

Goodman, L.J. (1970). The structure and function of the insect ocellus. Advances in Insect Physiology 7:97-195.

Graham, C.H. and Hartline, H.K. (1935). The response of single visual cells to lights of different wavelengths. J. of Gen. Physiol. 18:917-931.

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. 151:141-160.

Hartline, H.K. (1934). Intensity and duration in the excitation of single photoreceptor units. J. of Cellular and Comparative Physiology 5:229-247.

Hartline, H.K. (1940). The effects of spatial summation in the retina on the excitation of the fibers of the optic nerve. Am. J. Physiol. 130:700-711.

Hartline, H.K. and Graham, C.H. (1932). Nerve impulses from single receptors in the eye. J. of Cellular and Comparative Physiol. 1:227-295.

Hartline, H.K. and Macdonald, P.R. (1947). Light and dark adaptation of single photoreceptors in the Limulus eye. J. of Cellular and Comparative Physiol. 30: 225-253.

Hartline, H.K.; Wagner, H.G.; MacNichol, E.F. (1952). The peripheral origin of nervous activity in the visual system. Cold Spring Harbour Symposium on Quantitative Biology 17:17-36.

Hollenberg, M.D. (1978). Receptor models and the action of neurotransmitters and hormones. In: Neurotransmitter Receptor Binding, Yamamura, H.I.; Enna, S.J.; Kuhar, M.J. (Eds.). New York: Raven Press, 13-39.

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

Humason, G. L. (1979). In: Animal Tissue Techniques. W.H. Freeman and Company, San Francisco. pg 112, 118, 119-122.

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

Johnston, G.A.R. (1978). Neuropharmacology of Amino Acid inhibitory transmitters. Ann. Rev. Pharmacol. Toxicol. 18:269-289.

Kato, E.; Morita, K.; Yuba, K.; Yamada, S.; Kultora, T.; Shinko, T.; Matsomoto, I. (1980). Does GABA in blood control transmitter release in Bullfrog sympathetic ganglion? Brain Research 195:208-214.

Katz, B. (1950). Depolarization of sensory terminals and the initiation of impulses in the muscle spindle. J. Physiol. (London) 111:261-282.

Katz, B. (1962). The transmission of impulses from nerve to muscle, and the subcellular unit of synaptic action. Proc. Roy. Soc. B 155:455-477.

Katz, B. and Miledi, R. (1965). The measurement of synaptic delay, and the time course of acetylcholine release at the neuromuscular junction. Proc. Roy. Soc. B 161:483-495.

Katz, B. and Miledi, R. (1967). The release of acetylcholine from nerve endings by graded electric pulses. Proc. Roy. Soc. B 167:23-38.

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.

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

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

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

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

Kolb, H. and Famiglietti, E.V. (1974). Rod and cone pathways in the inner plexiform layer of cat retina Science 186:47-49.

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

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

Lew, G.T. (1933). Head characters of the Odonata with special reference to the development of the compound eye. Entomological America 14:41.

Miller, J.J. (1979). The neuronal basis of ganglion cell receptive field organization and the physiology of amacrine cells. In The Neurosciences, Fourth Study Program, Schmitt, F.O. and Worden, F.G. (Eds.) Cambridge, Mass:MIT Press, 227-245.

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. 132:87-95.

Olsen, R.W.; Ticku, M.K.; Van Ness, P.C.; Greenlee, D. (1978). Effects of drugs on gamma-aminobutyric acid receptors, uptake, release, and synthesis, in vitro. Brain Research 139:277-294.

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.

Pappas, L.G.; Eaton, J.L. (1977). The internal ocellus of manduca sexta: electroretinograms and spectral sensitivity. J. Insect Physiol. 23:1355.

Parry, D.A. (1947). The function of the insect ocellus. J. Exp. Biol. 24:.

Patterson, J. and Chappell, R.L. (1979). Intracellular responses of procion filled cells and whole nerve cobalt impregnation in the dragonfly median ocellus J. Comp. Physiol. 139:25-39.

Patterson, J. and Goodman, L.J. (1975). Componental Analysis of the ocellar electroretinogram of the locust, Schistocerca gregaria. J. Insect Physiol. 21:287-298.

Pasztor, V.M. and Bush, B.M.H. (1982). Impulse-coded and analog signaling in single mechanoreceptor neurons. Science 215:1635-1637.

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.

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

Purkinje, J.E. (1819). Beobachtungen and versuche zur physiologie der sinne. 1. Beitrage zur kenntnis des sehens in subjektiver hinsicht. Calve, Prag.

Ratliff, F. (1974). Neural activity generated by single photoreceptor units in the eye of Limulus. "Studies on excitation and inhibition in the retina". Ratliff, F. Editor. Rockefeller University Press, New York.

Ratliff, F.; Hartline, H.K.; Miller, W.H. (1963). Spatial and temporal aspects of retinal inhibitory interaction. J. of the Optical Society of America 53: 110-120.

Robertson, R.M. and Moulins, M. (1981). Firing between two spike thresholds: implications for oscillating lobster neurons. Science 214:941-943.

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

Ruck, P. (1958). A comparison of the electrical responses of compound eyes and dorsal ocelli in four insect species. J. Insect Physiol. 2:261-274.

Ruck, P. (1961). Electrophysiology of the insect dorsal ocellus. I. Origin of the components of the electroretinogram. II. Mechanisms of generation and inhibition of impulses in the ocellar nerve of dragonflies. III. Responses to flickering light of the dragonfly ocellus. J. Gen. Physiol. 44:605-628, 629-639, 641-657.

Ruck, P. (1962). On Photoreceptor mechanisms of retinula cells. Biological Bull. 123:618-634.

Ruck, P. (1965). The components of the visual system of a dragonfly. J. Gen. Physiol. 49:287.

Ruck, P. (1966). Extracellular aspects of receptor excitation in the dorsal ocellus. In: Proceedings of the International Symposium on the Functional Organization of the Compound Eye. Oxford and New York:Pergamen Press, 195-205.

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

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.

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.

Stone, S. (1982). Electrophysiology and pharmacology of a local circuit feedback system in neurons of the ocellar retina. Ph.D thesis: City University of New York, New York.

Stone, S. and Chappell, R.L. (1981). Synaptic feedback onto photoreceptors in the ocellar retina. Brain Research 221:374-381.

Strausfeld, N.J. and Obermayer, M. (1970). Resolution of intraneuronal and transynaptic migration of cobalt in the insect visual and central nervous system. J. Comp. Physiol. 110:1-12.

Stange, G. and Howard, J. (1979). An ocellar dorsal light response in a dragonfly. J. Exp. Biol. 83: 351-355.

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

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

Ticku, M.K. and Olsen, R.W. (1978). Interaction of barbiturates with dihydropicrotoxin binding sites related to the GABA receptor-ionophore system. Life Sciences 22:1643-1652.

Tomita, T. (1968). Electrical responses of single photoreceptors. Proc. I.E.E.E. 56:1015.

Tyrer, N.M. and Bell, E.M. (1974). The intensification of cobalt filled neuron profiles using a modification of Timm's sulfide silver method. Brain Res. 73:151-155.

Walker, R.J.; Crossmen, A.R.; Woodruff, G.N.; Kerkut, G.A. (1971). The effect of bicuculline on the GABA receptors of neurons of Periplaneta americana and Helix aspersa. Brain Research 33:75-82.

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

Werblin, F. (1979). Integrative pathways in local circuits between slow-potential cells in the retina. In: The Neurosciences, Fourth Study Program, Schmitt, F.O. and Worden, F.G. (Eds.). Cambridge, Mass: MIT Press, 193-211.

Werblin, F. and Dowling, J.E. (1969). Organization of the retina of the mudpuppy, Necturus, maculosus. II. Intracellular recordings. J. Neurophysiology 32: 339-355.

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

Yaksh, T.L. and Tyce, G.M. (1980). Resting and K+ evoked release of serotonin and norepinephrine in vivo from the rat and cat spinal cord. Brain Research 192: 133-146.

Zenkin, G.M. and Piqarev, I.N. (1971). Optically determined activity in the cervical nerve chain of the dragonfly. Biofizika 16:299-306.

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

External anatomy of the dragonfly head

A drawing of the head of an adult Aeschna. (adapted from Lew, 1933; plate 13). compound eye (CE), lateral ocellus (LO), median ocellus (MO), vertex (VX), antenna (A), clypeus (CLP), frons (FR).

Figure 1

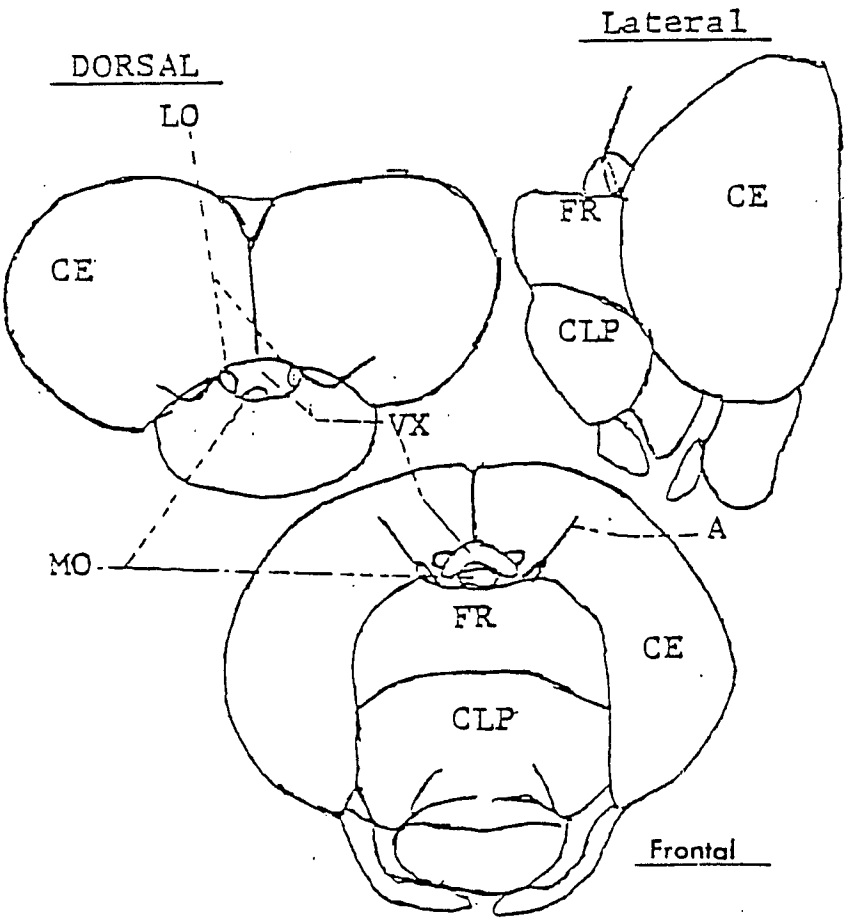


Figure 2

Histology of the dragonfly brain and ocelli.

A and B: a whole mount preparation of the median and right lateral ocelli, their nerves, and the brain obtained from an Aeschna. One can see the dendritic branching and nerve process of a neuron stained with cobalt via an intracellular electrode that was placed in the ocellar nerve (see methods). The light response of this cell can be seen in figure 12. The left lateral ocellus and its nerve were removed prior to the intracellular penetration. The cell appears to be one of the ipsilateral neurons described by Chappell, et. al., 1978.

C: A five micron thick parafin section of the dragonfly ocellar system (from a different animal). The slide was stained with Hematoxylin and Eosin using the technique described in Humason, 1979.

Median ocellar nerve (MON), lateral ocellar nerve (LON), median ocellus (MO), ocellar nerve dendrites (OND), lateral ocellus (LO), rhabdomeric region (R), photoreceptor nuclear layer (N), layer of pigmented epithelium (P), synaptic zone (S). Corneal lens is not present.

②

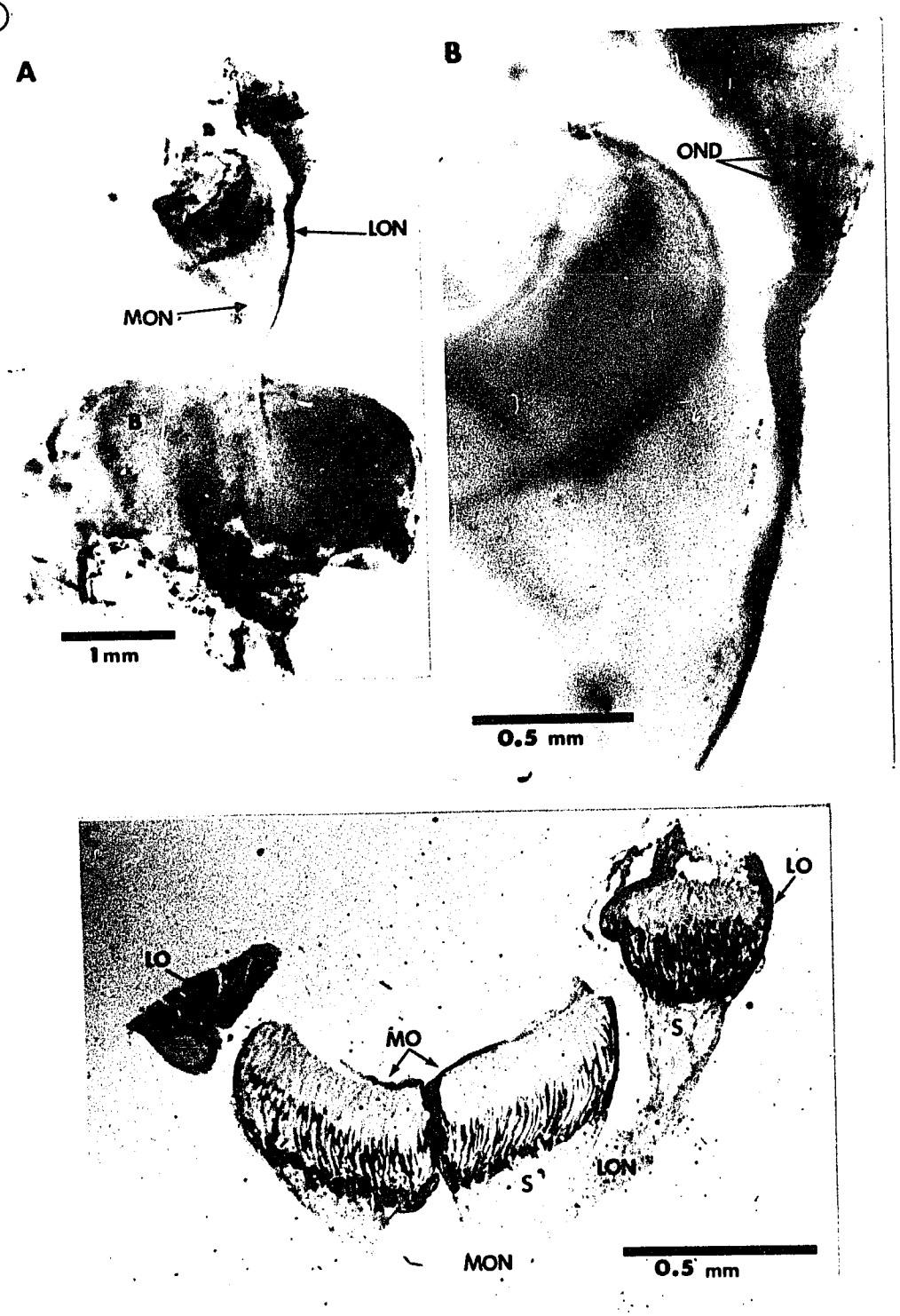


Figure 3

The configuration used to record impulse activity from the median ocellus.

A schematic diagram of the dragonfly ocelli, ocellar nerves, and brain showing placement of electrodes for extracellular recordings of ocellar nerve impulse activity from the median ocellus. Left lateral ocellus (LLO), right lateral ocellus (RLO), left lateral ocellar nerve (LLON), right lateral ocellar nerve (RLON), median ocellus (MO), median ocellar nerve (MON), G2 (electrode connected to the input G2 of the preamplifier), G1 (electrode connected to G1 of the preamplifier).

Figure 3

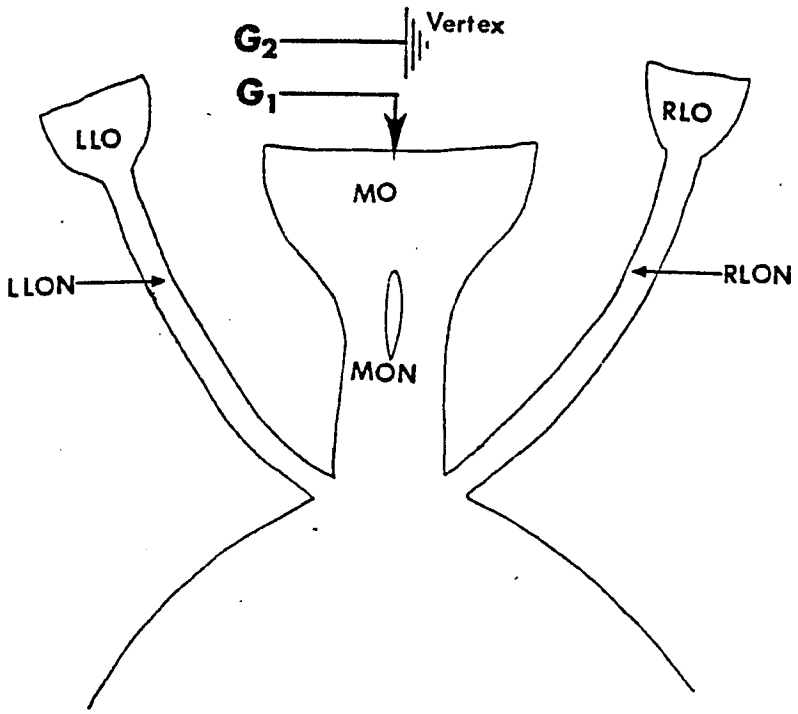


Figure 4

The impulse activity recorded from the median ocellus.

Extracellular recording from the cornea of the median ocellus. The electrode configuration used is shown in figure 3. The frequency bandpass for A, B, and C was 300 Hz to 1 KHz, and for D, 0.1 Hz to 100 Hz. The time at which the light was on is denoted by a downward deflection on the stimulus trace (E).

A: recording from the cornea of the median ocellus with all three ocelli and their nerves intact. Note the noisy appearance of the trace as compared to B.

B: recording after the left lateral ocellar nerve was removed. Large light-inhibited and small light-facilitated action potentials can be clearly distinguished.

C: recording after both lateral ocellar nerves were removed. The spikes at light "on" and light "off" are the highly filterd ERG from the median ocellus.

D: an ERG from the median ocellus. Note the presence of the of the small inflection on the rising phase of the light response. This is component 3, the electrical contribution of the ocellar nerve dendrites to the ERG waveform. The "off" response can be seen as the slight rise in the potential immediately after light "off" (see discussion for explanation). Stimulus intensity Log I= 0.

Figure 4

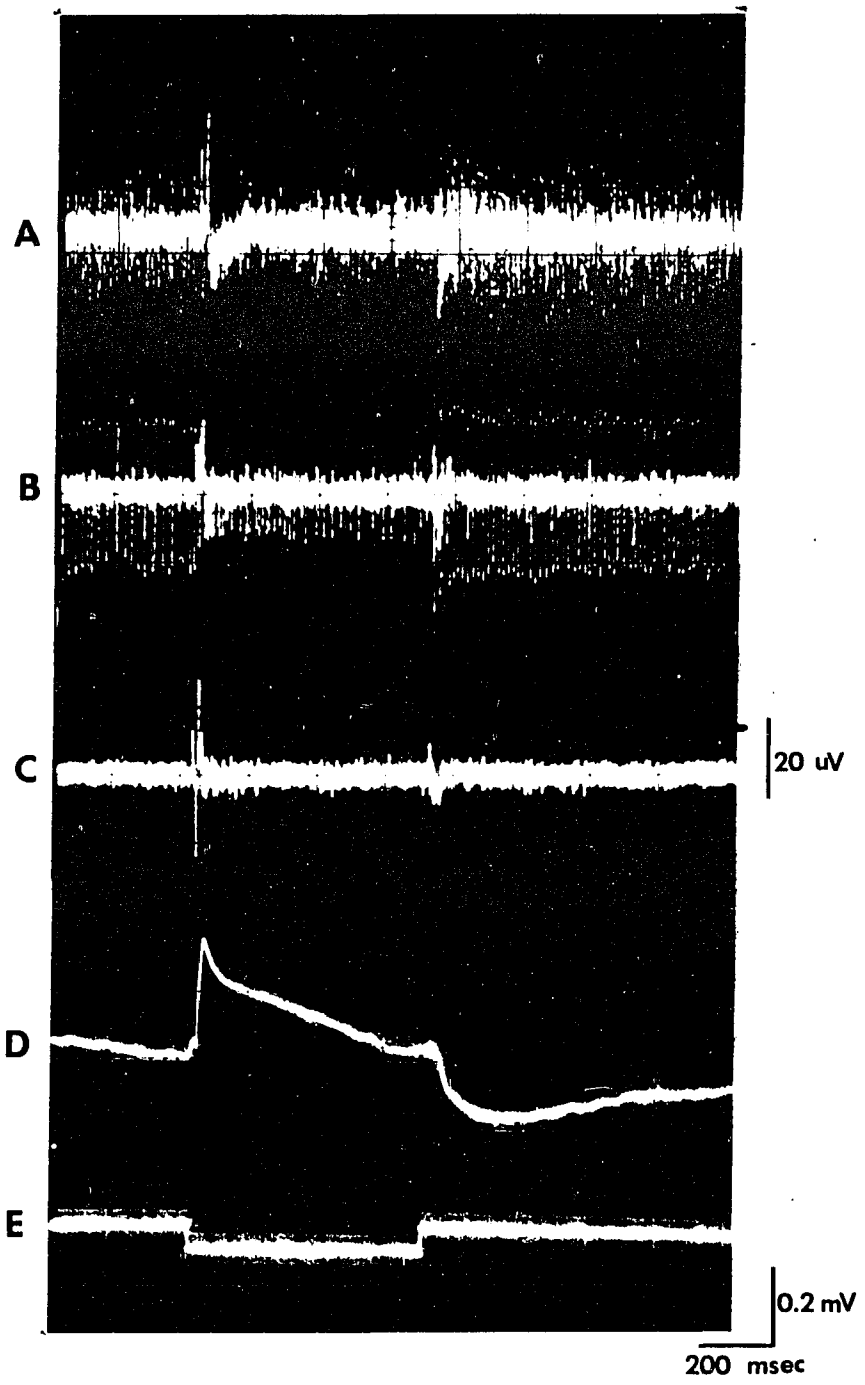


Figure 5

The configuration for recording impulse activity from the lateral ocelli.

A schematic diagram of the dragonfly ocelli, ocellar nerves and the brain, showing placement of electrodes for differential recordings between the corneas of the right and left lateral ocelli. left lateral ocellus (LLO), right lateral ocellus (RLO), left lateral ocellar nerve (LLON), right lateral ocellar nerve (RLON), median ocellus (MO), median ocellar nerve (MON), G2 (electrode connected to input G2 of the preamplifier), G1 (electrode connected to input G1 of the preamplifier).

Figure 5

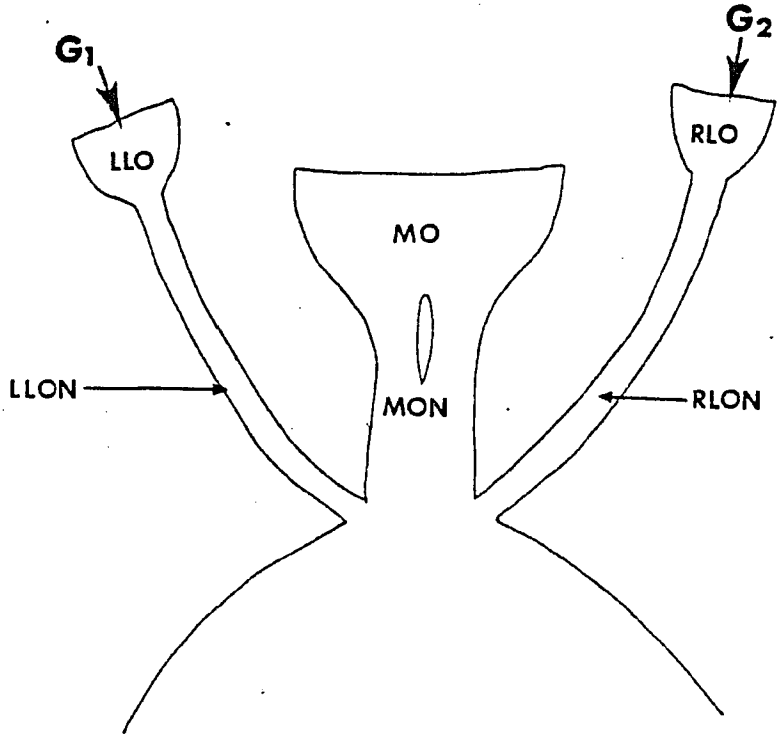


Figure 6

Ocellar nerve impulse activity recorded from the lateral ocelli.

Differential recordings between the corneas of the lateral ocelli (see "Results:Origin of ocellar impulse activity" for further explanation). Recording configuration is shown in figure 5. All recordings were made after the extirpation of the median ocellus, antennal nerves, optic lobes and cervical neck chain. Note the decrease in noise and the increase in the amplitude of the large spikes after the left ocellar nerve was ablated. Both the large light inhibited and small light facilitated action potentials are clearly discernible in both the upper and middle traces. Also the frequency of spikes is diminished in the middle trace as compared to the upper trace. The spike at light "on" of the lower trace may be due to the ERG of the lateral ocelli. light intensity Log I= 0.

Figure 6

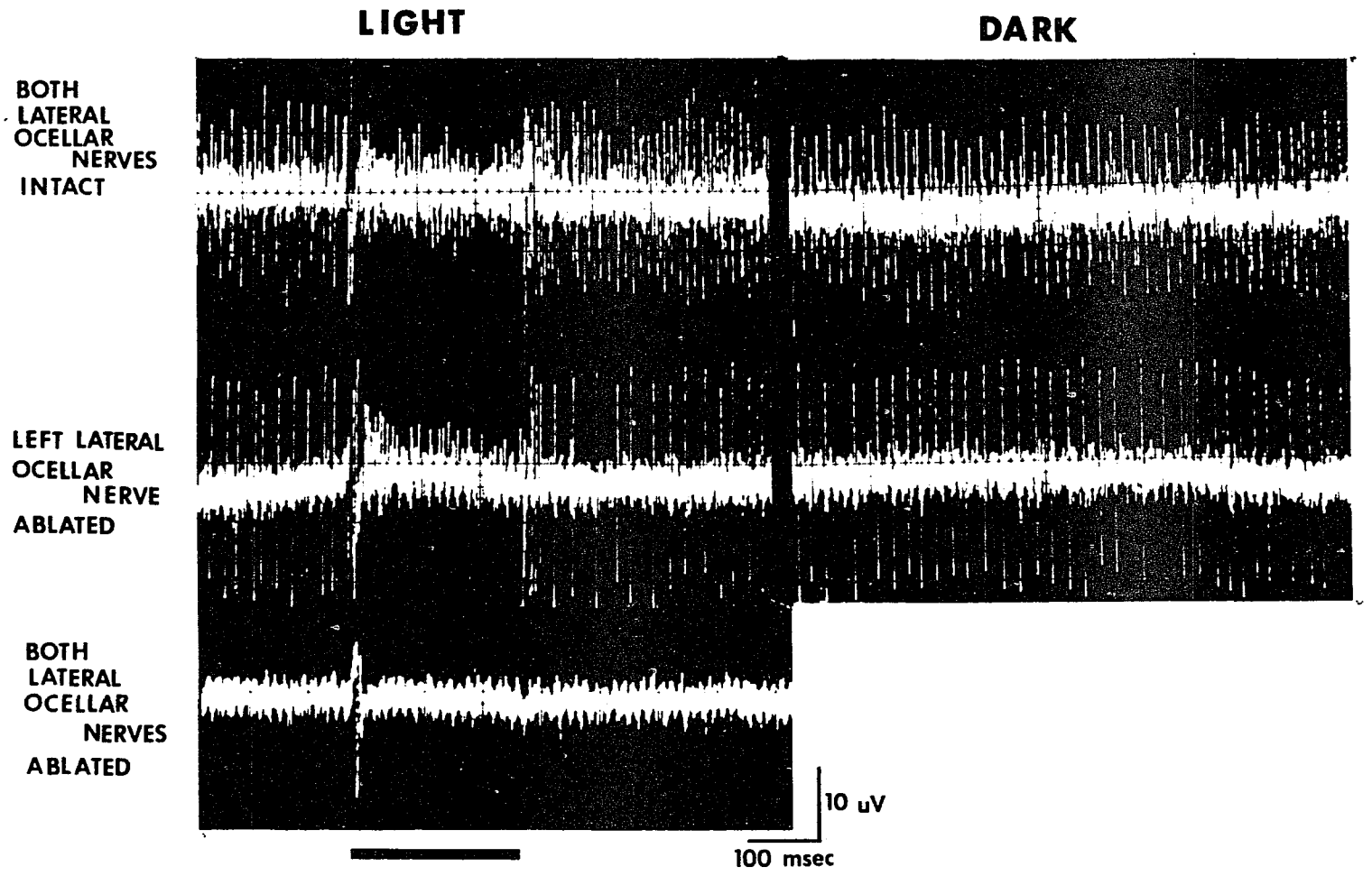

LIGHT
DARK
BOTH LATERAL OCELLAR NERVES INTACT
LEFT LATERAL OCELLAR NERVE ABLATED
BOTH LATERAL OCELLAR NERVES ABLATED
10 uV
100 msec

Figure 7

Ocellar nerve impulse activity in the dark

The same recordings of dark spikes as in figure 6, on an expanded time scale, show that there are spikes of two (opposite) polarity sequences present prior to ablation of one of the lateral ocellar nerves. There was a decrease in noise after the left lateral ocellar nerve was ablated (lower trace) and spikes with only one polarity sequence remain. Also, there was an increase in size of the remaining spikes.

Figure 7

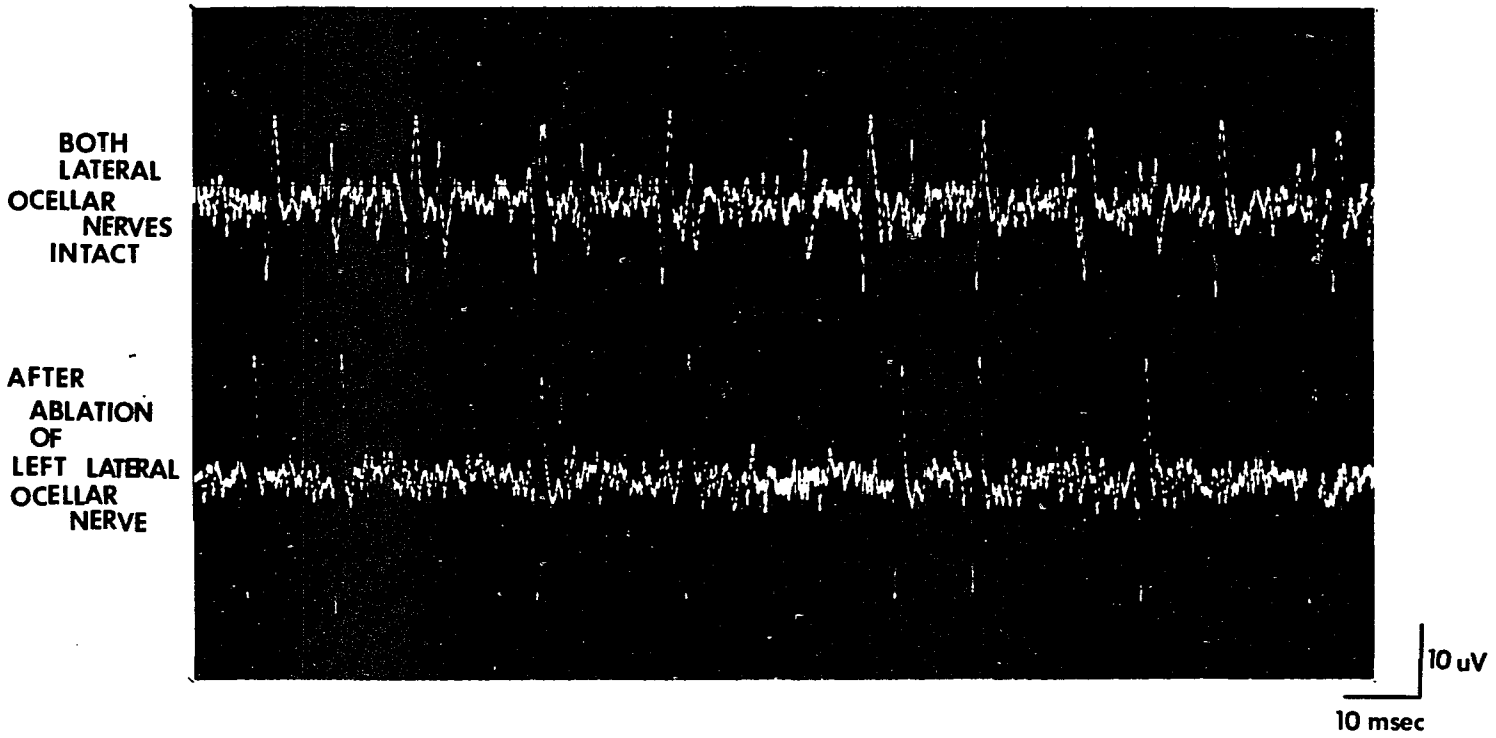

BOTH LATERAL OCELLAR NERVES INTACT
AFTER ABLATION OF LEFT LATERAL OCELLAR NERVE
10 uV
10 msec

Figure 8

Simultaneous extracellular recordings

A schematic diagram of the dragonfly ocelli, ocellar nerves and the brain, showing the electrode configuration used to record differentially between the corneas of the lateral ocelli at the same time that extracellular recordings were made from the cornea of the median ocellus. Right lateral ocellar nerve (RLON), left lateral ocellar nerve (LLON), left lateral ocellus (LLO), right lateral ocellus (RLO), median ocellus (MO), median ocellar nerve (MON), preamp A G1 (electrode attached to input G1 of preamp A), preamp A G2 (electrode attached to input G2 of preamp A), preamp B G1 (electrode attached to input G1 of preamp B), preamp B G2 (electrode attached to input G2 of preamp B).

Figure 8

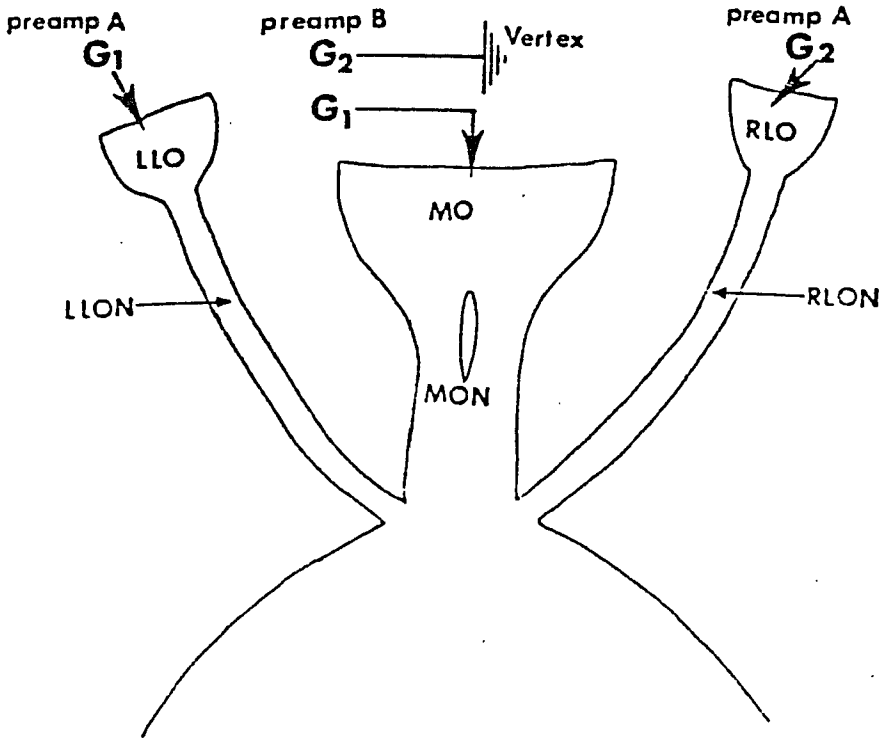

preamp A
G1
LLO
LLON
preamp B
G2
Vertex
G1
MO
MON
preamp A
G2
RLO
RLON

Figure 9

Simultaneous extracellular recordings.

The top trace of each pair of recordings is from the cornea of the median ocellus and was obtained at the same time as the differential recordings between the corneas of the lateral ocellus (seen in the lower trace). All spikes in the median ocellar recordings have a single orientation. The impulse activity recorded from the lateral ocelli, prior to the removal of either lateral nerve (A and D) shows spikes with two orientations. There appears to be a one to one correspondence between the spikes seen in the upper and lower traces. B and E: After the ablation of the left lateral ocellar nerve, action potentials with a single orientation are left in the lower trace. C and F: After the removal of the right lateral ocellar nerve, no spikes are left in either trace.

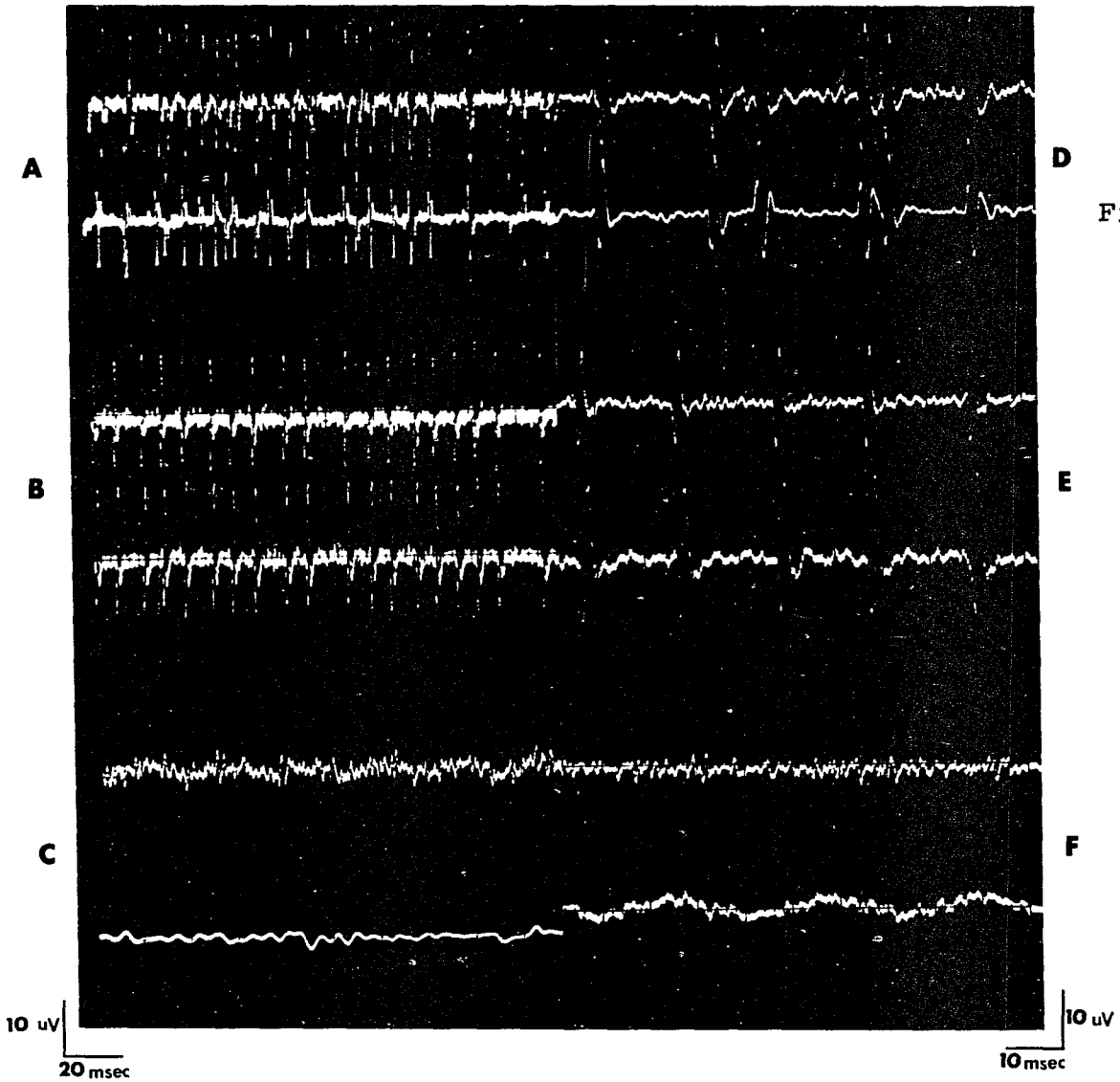



Figure 9

Figure 10

Simultaneous appearance of extracellular ocellar nerve impulses.

Recording electrodes as in figure 9. There does not appear to be any delay between the impulses recorded between the cornea of the median ocellus and vertex (top trace A, B, and C) and those recorded between the lateral ocelli (bottom trace A, B, and C,). It can be seen that the impulses in the top traces were always oriented with an initial negative wave no matter what the orientation of the impulse seen in the lower trace. Note expanded time scale for B and C.

Figure 10

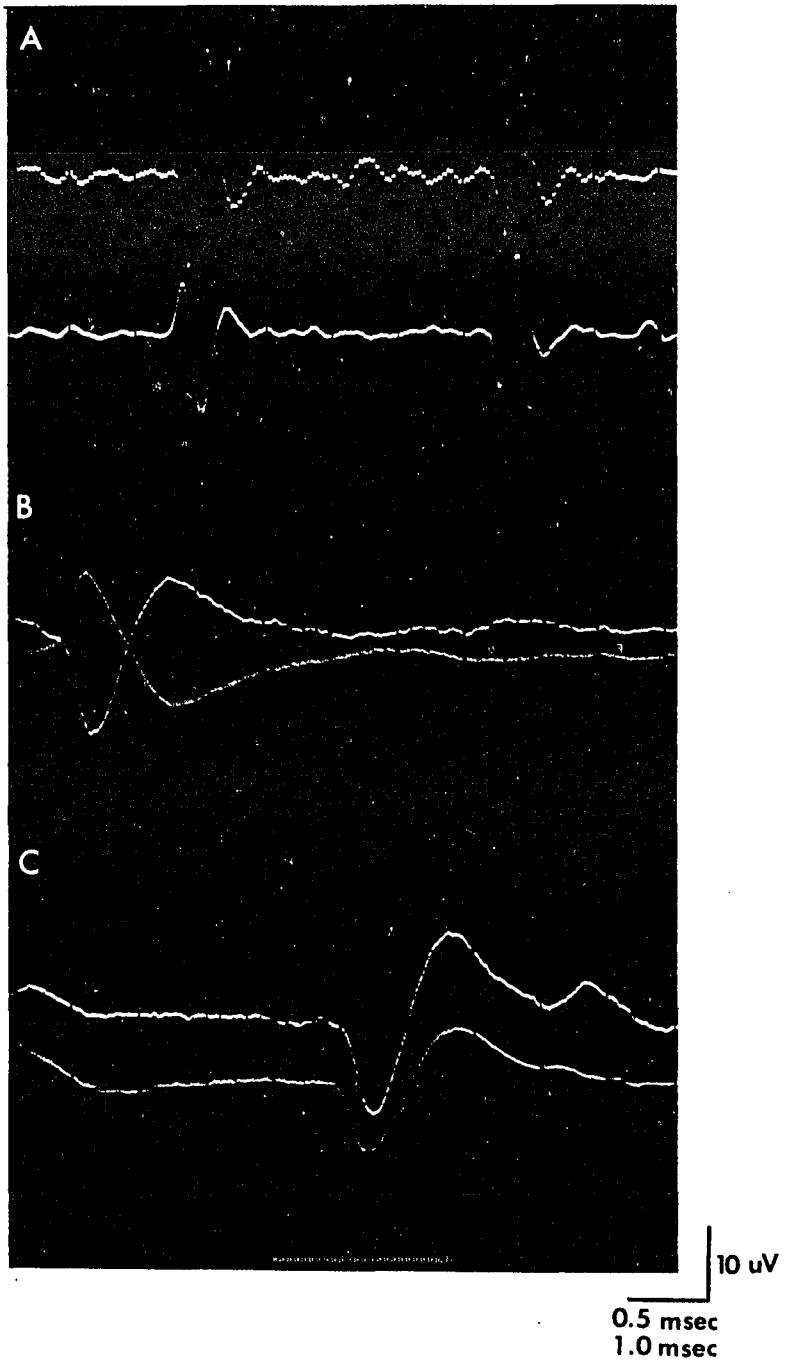


Figure 11

Intracellular recordings

Intracellular recordings from the lateral ocellar nerve showing a spiking (upper trace A and B) and non-spiking (middle trace A and B) cell. A downward deflection on the lower trace (A and B) indicates the time of illumination. The recordings of the two types of cells were obtained from different animals both of which were Aeschna. The resting potential of the spiking neuron was -55 mv and that of the non-spiking cell was -35 mv. stimulus intensity was log I=0.

Figure 11

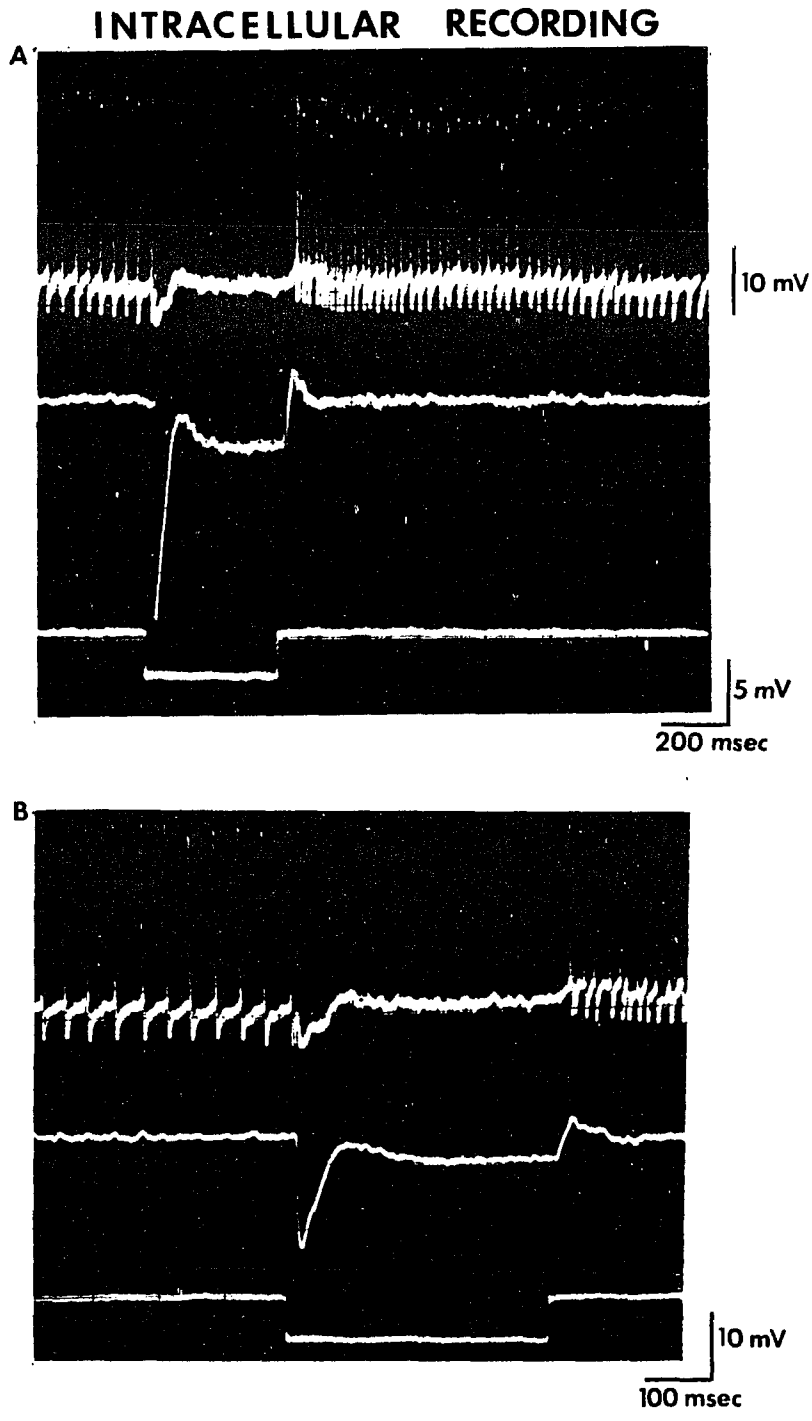


Figure 12

Simultaneous extracellular and intracellular recordings from the right lateral ocellar nerve.

The extracellular recording electrodes were placed as shown in figure 5. The intracellular recording was obtained from a microelectrode located at a point on the nerve midway between the ocellar cup and the brain using G2 as a reference. After recording the response, cobalt was introduced into the cell via the intracellular electrode. The cell can be seen in a whole mount preparation in figure 2. The resting potential for the cell was -30 mv.

Figure 12

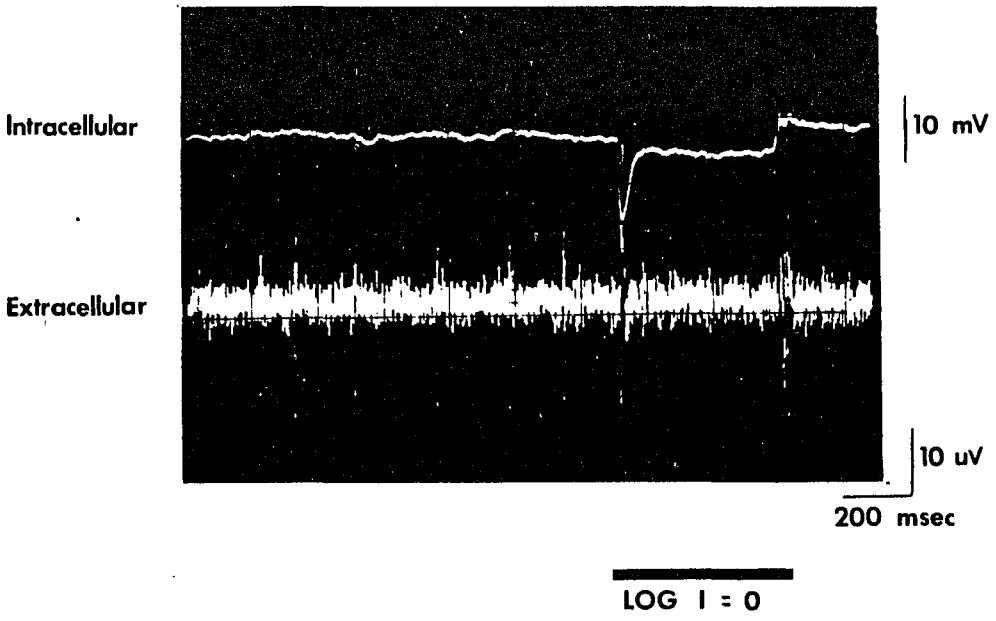


Figure 13

Intensity-frequency relationship of the impulse activity during a flash.

Vertical bars indicate standard deviation. Each animal received three two-second flashes at each intensity, each flash of light coming thirty-two seconds after the preceding one. Data from the first flash was not used. For each animal, the frequency for each half second interval during the last 1.5 seconds of the second and third flash was averaged at every intensity (see Methods: Pharmacology, curare). The mean frequency was plotted for 7 animals.

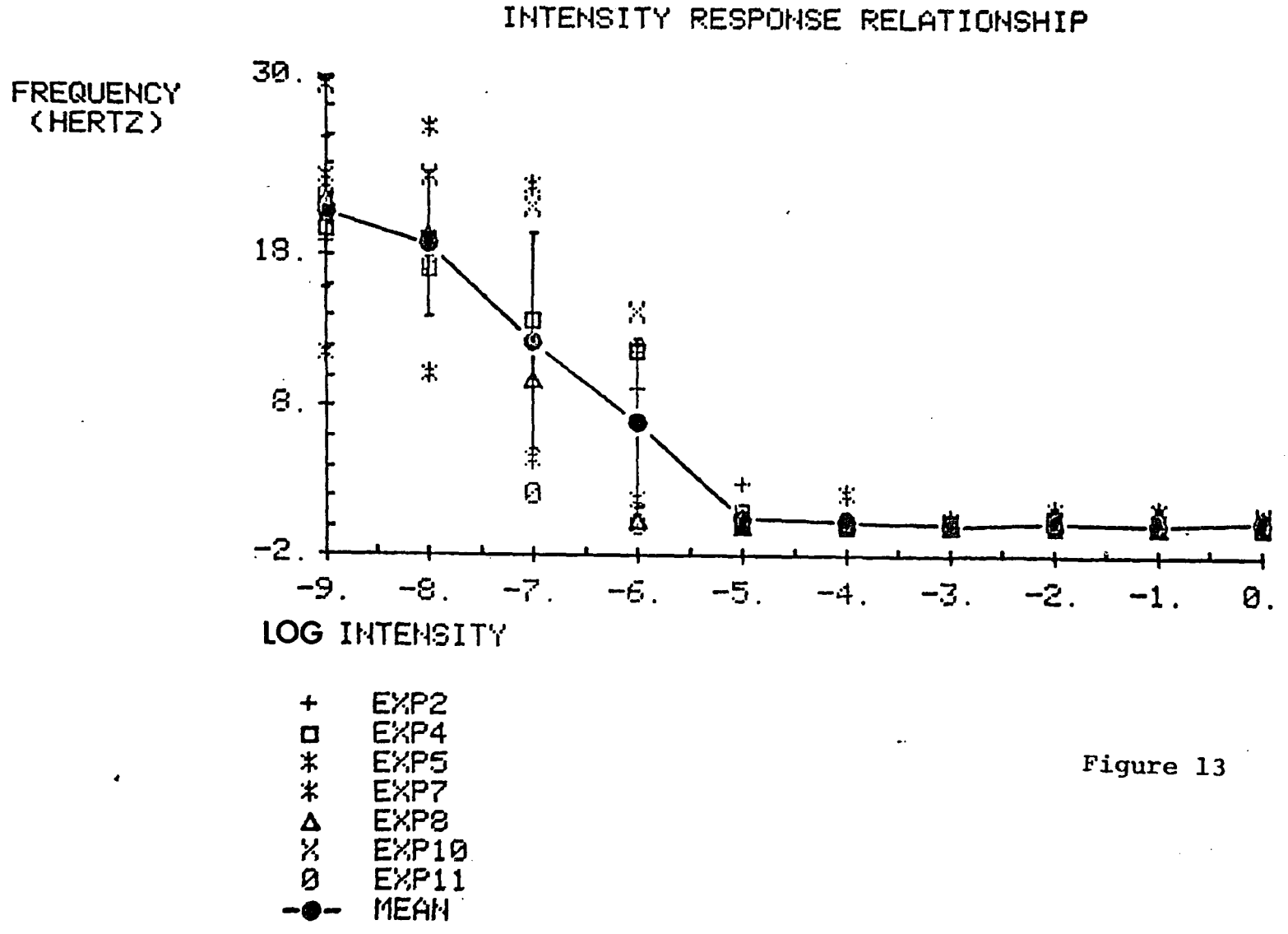

INTENSITY RESPONSE RELATIONSHIP
FREQUENCY
(HERTZ)
30.
18.
8.
-2.
-9.
-8.
-7.
-6.
-5.
-4.
-3.
-2.
-1.
0.
LOG INTENSITY
EXP2
EXP4
EXP5
EXP7
EXP8
EXP10
EXP11
MEAN

Figure 13

Figure 14

Effects of green and UV light.

Green and UV light intensity-response curves from threshold to saturation (complete inhibition). The curves are statistically parallel at $p>0.05$. The crosses and triangles represent the percent decrease in frequency in the last second of a 1.5 second light flash, for green and UV light respectively. The parameter was calculated in the following manner :
((frequency in the second preceding the stimulus) - (frequency during the flash) / (frequency in the second preceding the flash)) x 100. The frequency measurments were obtained from a single Aeschna.

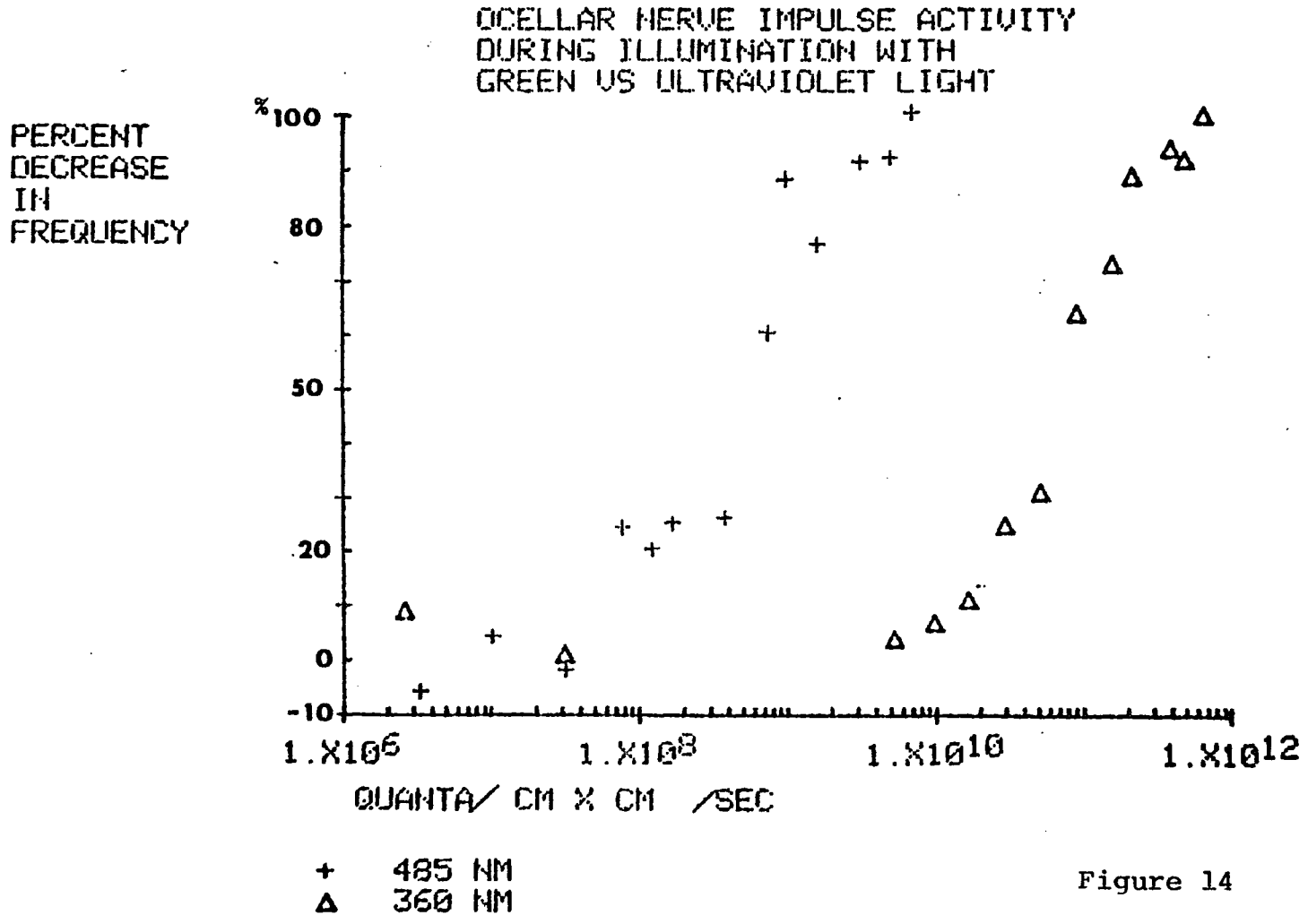

OCELLAR NERVE IMPULSE ACTIVITY
DURING ILLUMINATION WITH
GREEN VS ULTRAVIOLET LIGHT
PERCENT
DECREASE
IN
FREQUENCY
%100
80
50
20
0
-10
1.X10^6
1.X10^8
1.X10^10
1.X10^12
QUANTA/ CM X CM /SEC
+ 485 NM
Δ 360 NM

Figure 14

Figure 15

Tetrodotoxin

The effects of 0.3E-6 molar tetrodotoxin on spontaneous impulse activity. Within 30 seconds after the start of the drug perfusion the size of the action potentials was significantly decreased (A and on expanded time scale in B). After 3 minutes, all spiking activity had dissappeared (A). One hour of washing in Ringer did not reverse the effects of the drug. Electrodes were placed as shown in figure 5.

Note: E = exponential

for example 1.0E3 = 1000.0

and 1.0E-3 = 0.001

Figure 15

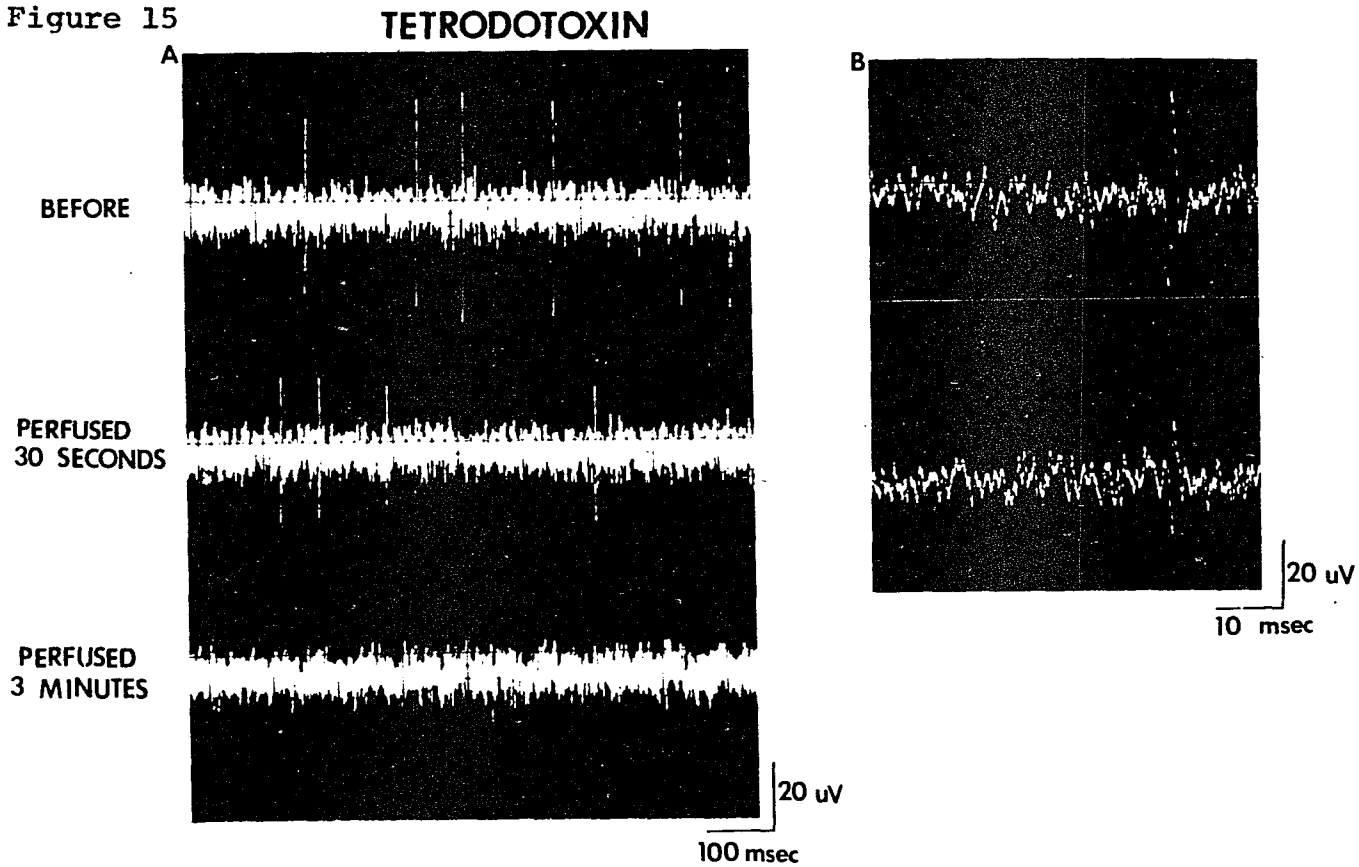

TETRODOTOXIN
A
BEFORE
PERFUSED
30 SECONDS
PERFUSED
3 MINUTES
20 uV
100 msec
B
20 uV
10 msec

Figure 16

Tetrodotoxin

The effects of 0.3 E-6 molar tetrodotoxin on impulse activity. This is a different animal than that shown in the previous figure. Middle trace: impulse activity has dissappeared after 5 minutes of perfusion with the drug. Lower trace: a normal DC ERG obtained after impulse activity had ceased. The recordings were made with electrode G1 in the cornea of the right lateral ocellus and electrode G2 in the vertex.

Figure 16

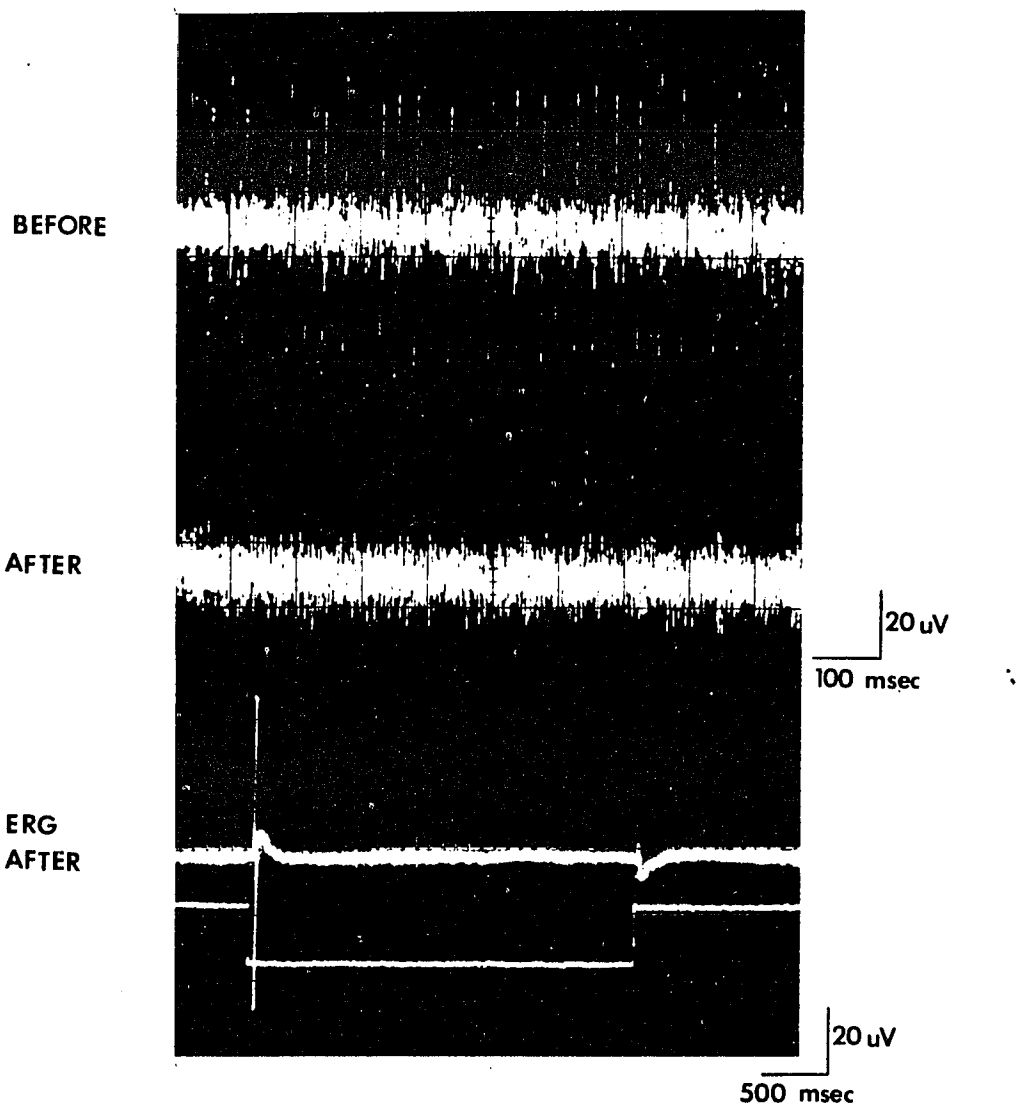

TETRODOTOXIN
BEFORE
AFTER
ERG
AFTER
20 uV
100 msec
20 uV
500 msec

Figure 17

Computers ability to count impulses.

Extracellular recordings of impulse activity. The electrode configuration as in figure 5. The line of dots above the impulse activity is a recording of the computers' discriminator output(see Methods: pharmacology, curare). For every impulse that is actually counted by the computer, the output of the discriminator puts out a positive voltage which can be displayed on an oscilloscope along with the signal which is being analyzed. The type of display seen in this figure was used to set the level at which each spike was counted. As a further control some responses were hand counted and these numbers were compared to the counts obtained by the computer. Hand counts were identical to computer counts.

Figure 17

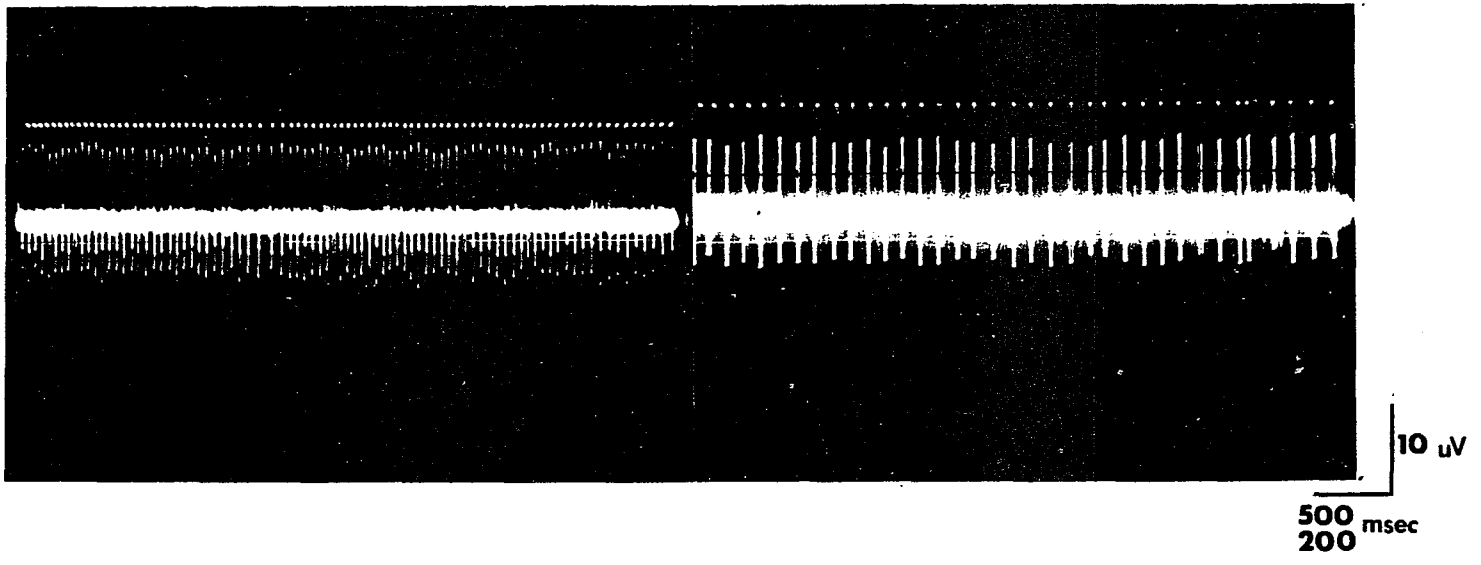


Figure 18

Intensity-Response series in Ringer

Intensity-response series showing typical behavior of ocellar nerve impulse activity before, during and after a two second light flash, and prior to the addition of any pharmacological agent. The recording electrodes were placed as described in figure 5 and the methods. One lateral ocellar nerve was removed. Complete inhibition of impulse activity occurs at light intensities of log I = -5 or greater. Note that the spike frequency prior to light "on" decreases with increasing stimulus intensity. Since these responses are either the second or third flash in a series of three flashes, the impulse rate just prior to the light flash represents the interflash spike frequency. At the higher light intensities a burst of impulse activity can be observed at light "off". The large downward spike present at light "on" at the two brightest stimulus intensities is a remnant of the corneal ERG, which is not completely filtered out. The responses displayed in figures 18 through 26 were all obtained from an Aeschna in a single experiment. The analyzed results shown in figures 29 through 34 came from 7 separate preparations, which includes the responses displayed in this series of figures (18-26).

Figure 18

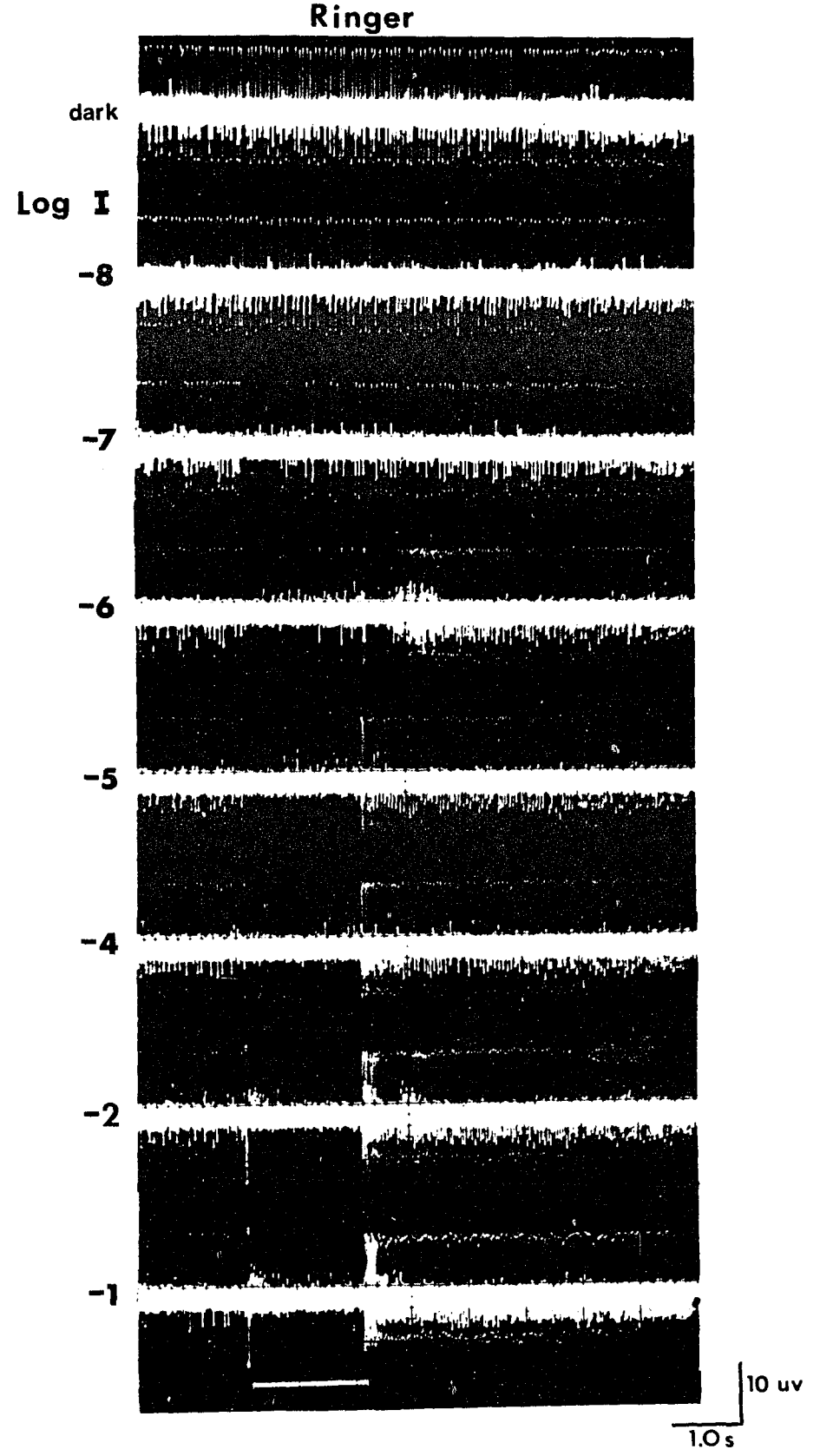


Figure 19

Curare

Intensity- response series showing the behavior of impulse activity in the presence of 50 uM curare. Compare the effect of a light flash at log I=-8 on the interflash spike frequency (frequency prior to stimulation) with the same trace in figure 18. Bar indicates time of illumination.

Figure 19

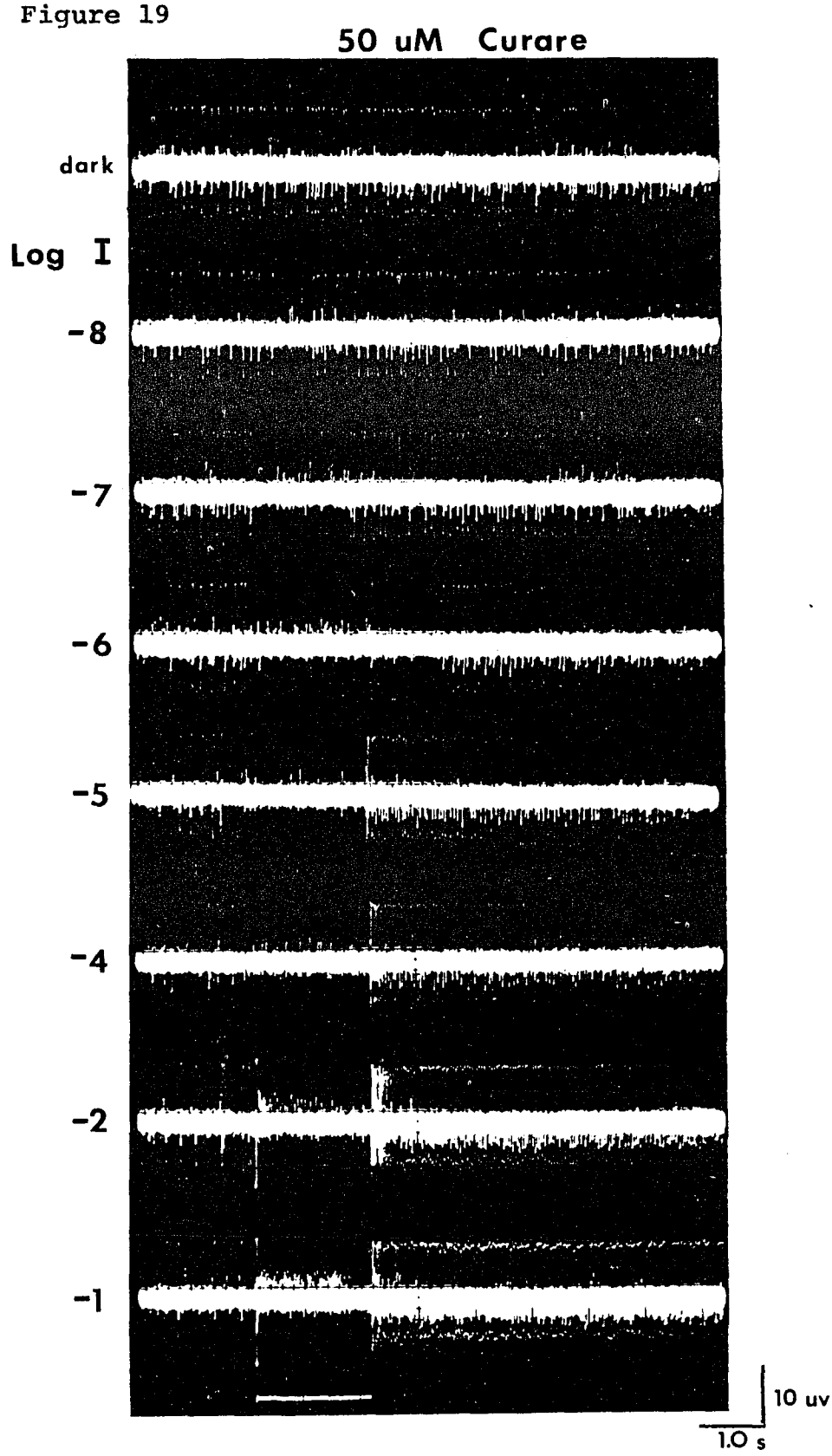


Figure 20

Curare

Intensity-response series showing the behavior of impulse activity in the presence of 500 um curare. There is an increase in frequency in the dark and during the end of the interflash interval (prior to the light flash) in response to this drug. Note the decrease in sensitivity of the light response. Bar indicates time of illumination.

Figure 20

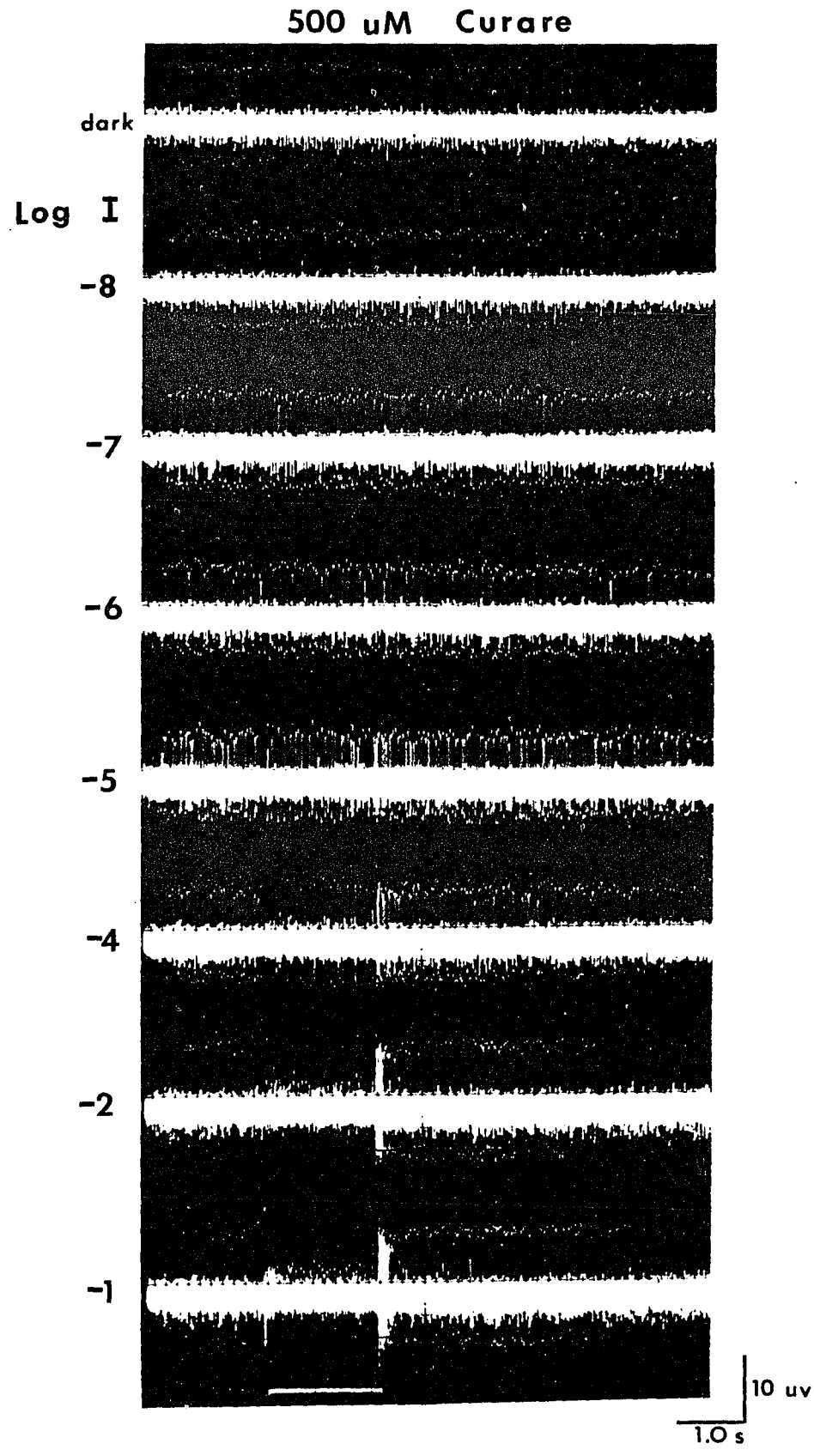


Figure 21

Curare

Intensity-response series showing the behavior of ocellar nerve impulse activity in the presence of 1.0 mM curare. Bar indicates time of illumination.

Figure 21

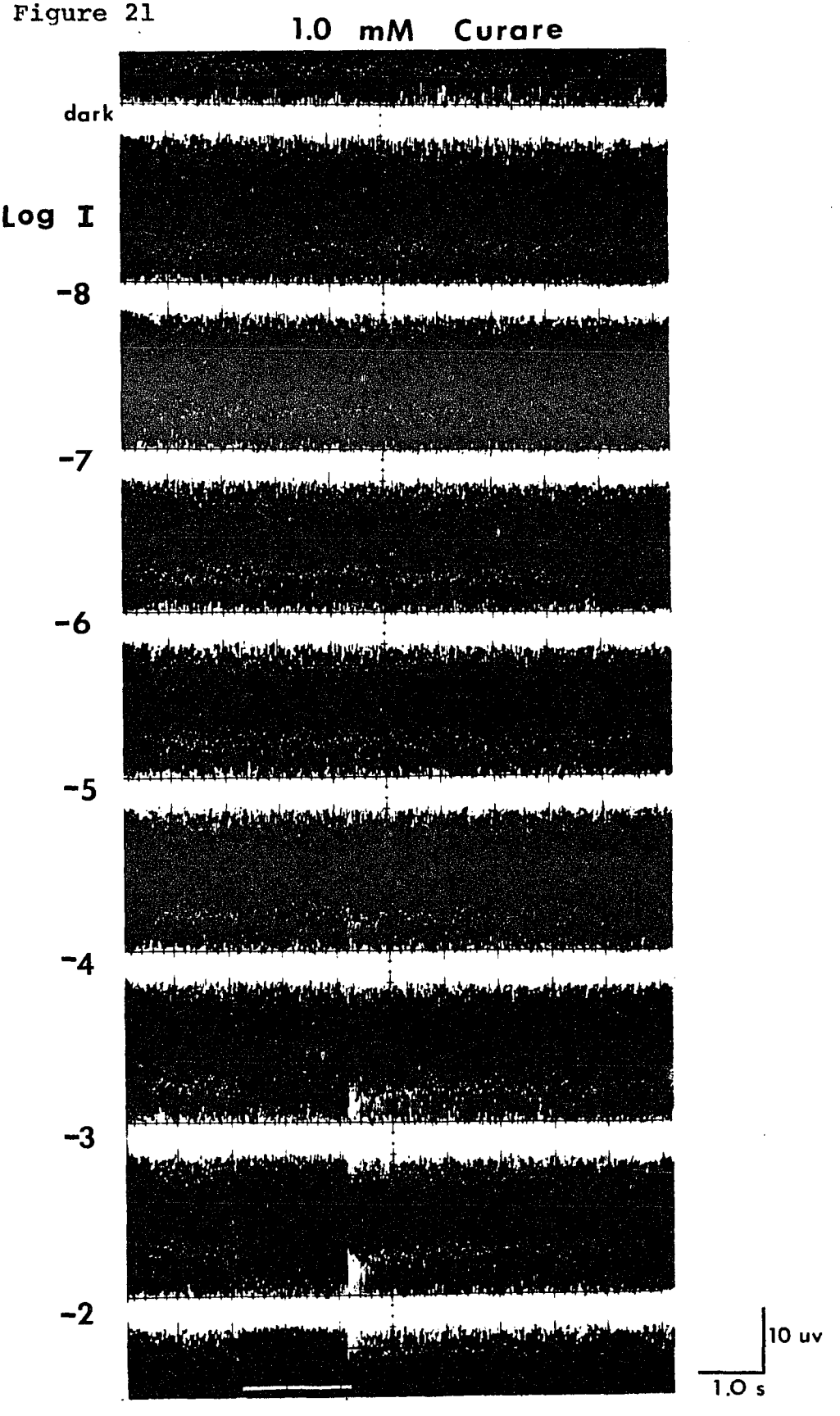


Figure 22

Curare

The effects of curare on ocellar nerve impulse behavior in the dark (A) and before, during, and after a light flash at three different intensities (B,C,D). Upper trace: recordings made in control perfusate...Ringer. Trace second from the top: impulse activity recorded in the presence of 50 uM curare. Trace third from the top: impulse activity recorded in the presence of 500 uM curare. Bottom trace: impulse activity recorded in the presence of 1.0 mM curare. Bar indicates time of illumination.

# Figure 22

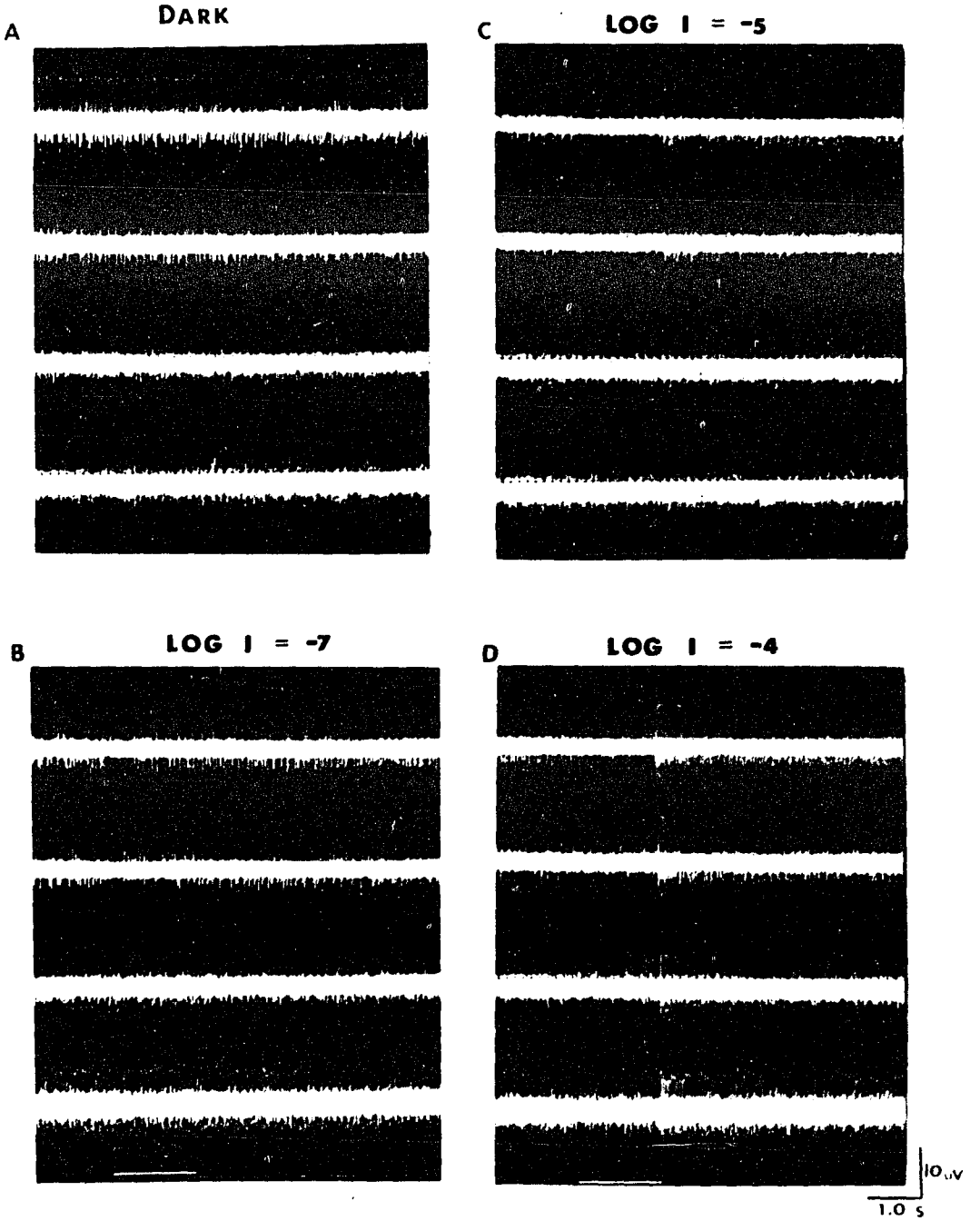


Figure 23

Histograms of spike frequency in the presence of Ringer.

A series of histograms showing the number of impulses per time interval starting at time zero seconds on the left. Each bar represents the number of counts for a period or interval of exactly 0.5 seconds. The time of illumination is denoted by the vertical dotted lines. This series of histograms is a computer representation of the response seen in figure 18. Note that figure 18 shows only the first 10 seconds of time displayed in figure 23.

Fig. 23

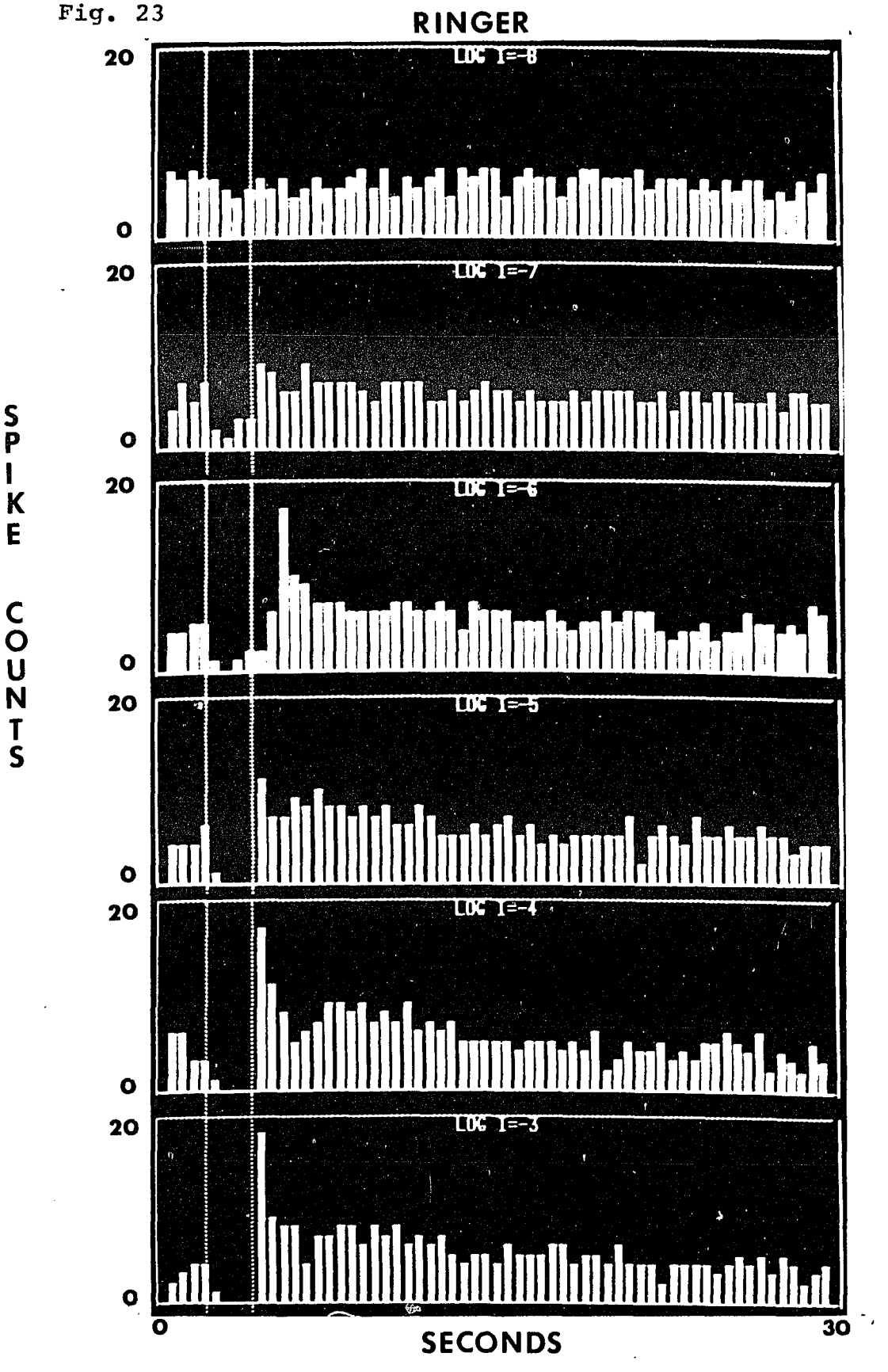

RINGER
LOC I=-8
LOC I=-7
LOC I=-6
LOC I=-5
LOC I=-4
LOC I=-3
20
0
SPIKE COUNTS
0
30
SECONDS

Figure 24

Histograms of spike frequency in the presence of 100 uM curare.

A series of histograms showing the effects of 100 uM curare on spiking activity. This was a unique preparation in that 100 uM curare further decreased spike frequency (see discussion:pharmacology) between flashes. This set of data was obtained from the same animal whose responses are seen in figures 18 through 26. The illumination occurred between the vertical lines.

Fig. 24

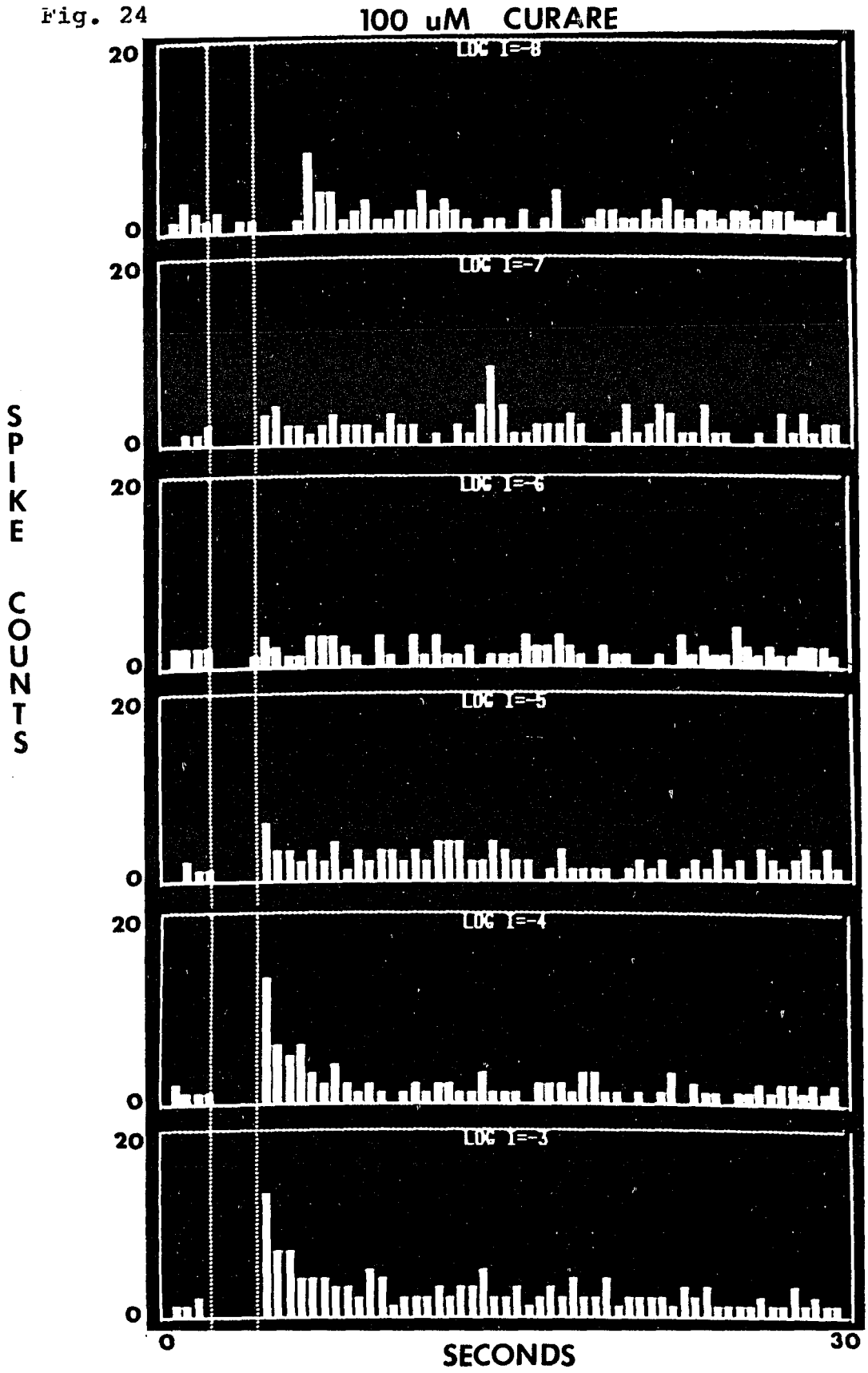

100 uM CURARE
LOG I=-8
LOG I=-7
LOG I=-6
LOG I=-5
LOG I=-4
LOG I=-3
20
0
SPIKE COUNTS
0
30
SECONDS

Figure 25

Histograms of spike frequency in the presence of 500 uM curare.

A series of histograms showing the effects of 500 uM curare on ocellar nerve impulse activity. This series is a computer representation of the responses seen in figure 20. The time of illumination is denoted by the vertical lines. Note the Y-axis of the bottom-most histogram has a different ordinate scale with an upper boundary of 30.

Fig. 25

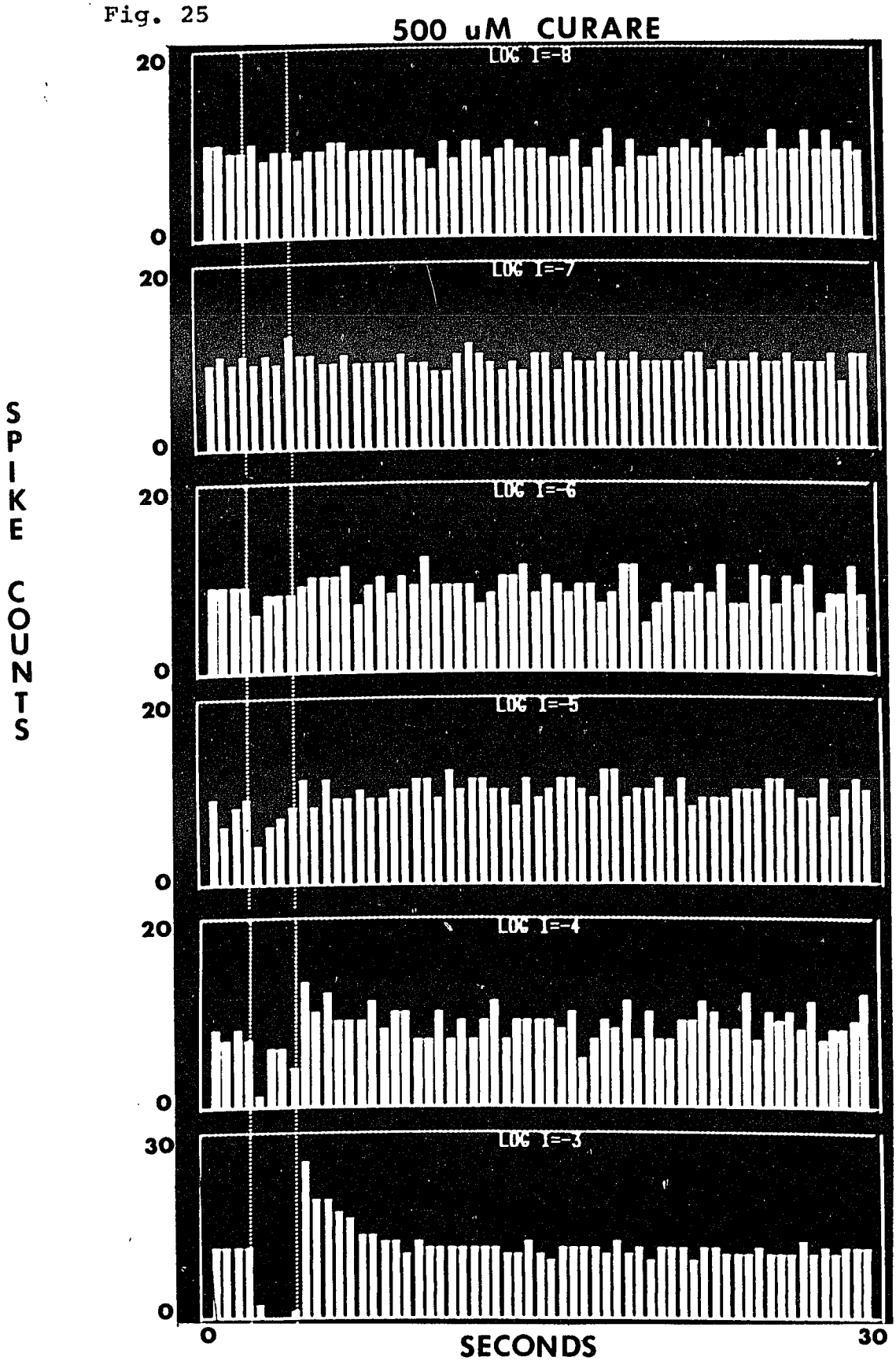

500 uM CURARE
LOG I=-8
LOG I=-7
LOG I=-6
LOG I=-5
LOG I=-4
LOG I=-3
20
0
20
0
20
0
20
0
20
0
30
0
SPIKE COUNTS
0
SECONDS
30

Figure 26

Histograms of spike frequency in the presence of 1.0 mM curare.

A series of histograms showing the effects of 1.0 mM curare on ocellar nerve impulse activity. These histograms are a computer representation of the responses seen in figure 21. The illumination occurred between the vertical bars.

Fig. 26

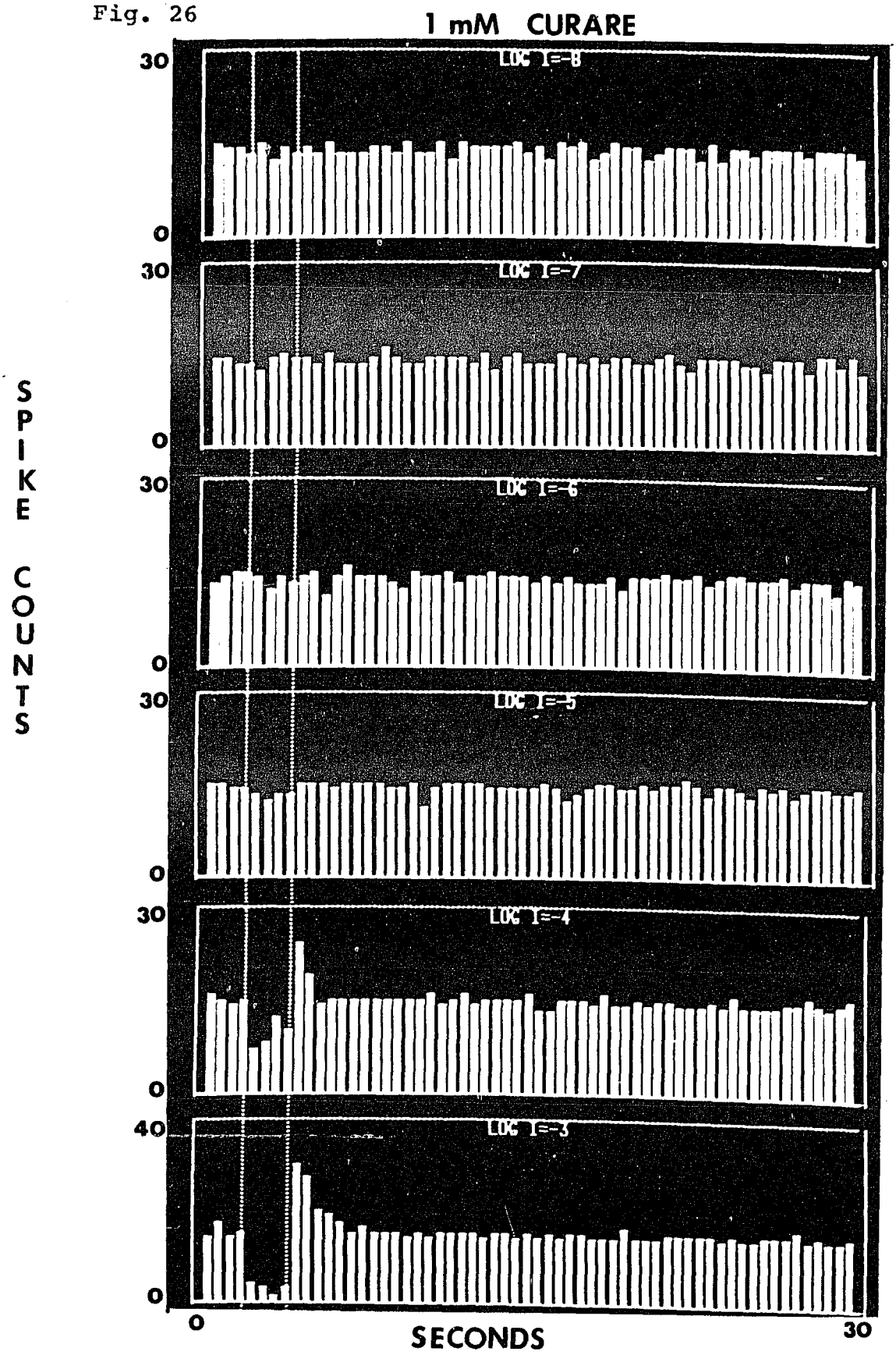

1 mM CURARE
LOC I=-8
LOC I=-7
LOC I=-6
LOC I=-5
LOC I=-4
LOC I=-3
30
0
30
0
30
0
30
0
30
0
40
0
SPIKE COUNTS
0
SECONDS
30

Figure 27

The reversibility of curare action.

The effects of 1.0 mM curare on impulse activity. The upper boundary of all histograms is 30 spikes. The time of illumination is denoted by the vertical bars. Curare severely decreased the response to light and increased spiking activity in the dark between flashes. The effects of curare are partially reversed after an hour in a Ringer wash.

## CURARE

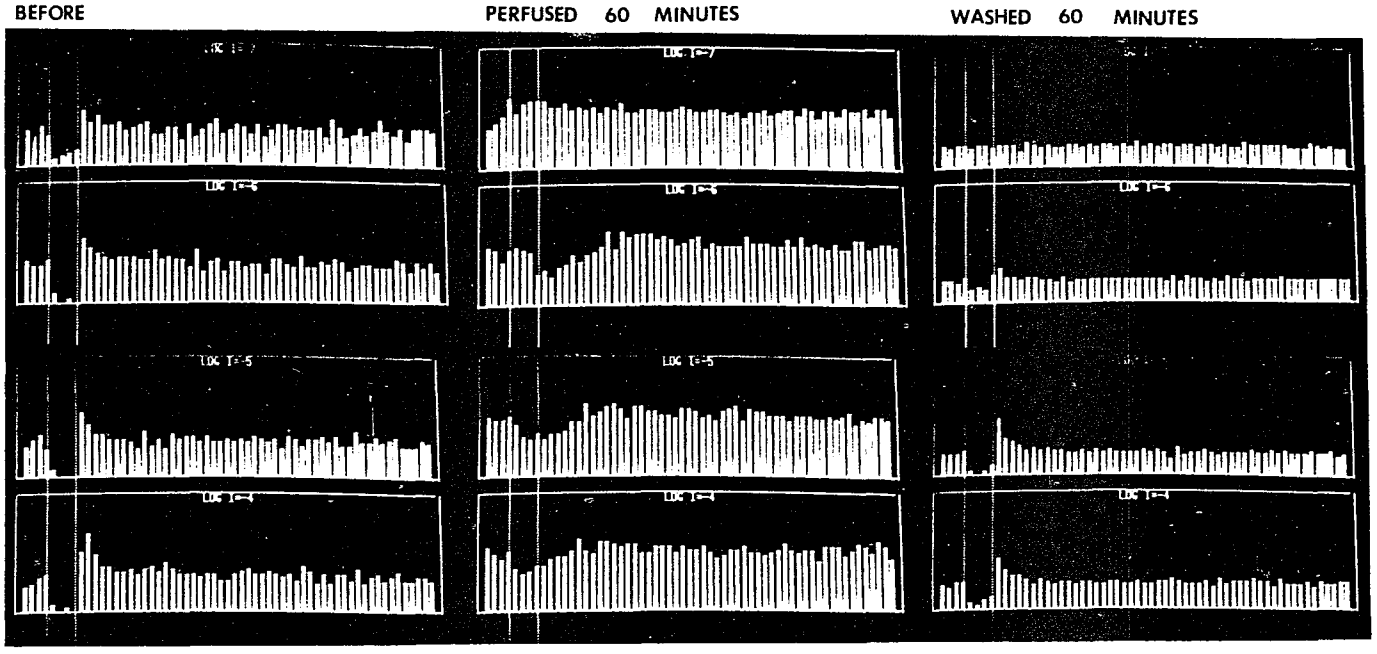



Figure 27

Figure 28

Impulse activity after two hours in Ringer.

There is no significant change in the behavior of the spiking activity after two hours in Ringer. A. A series of histograms showing the typical behavior of impulse activity after one hour in Ringer. B. As a control, this series of responses were repeated one hour after the first series (in A) was obtained. Time of illumination is denoted by the vertical lines. The upper boundary of the y-axis for all histograms is 20 spikes.

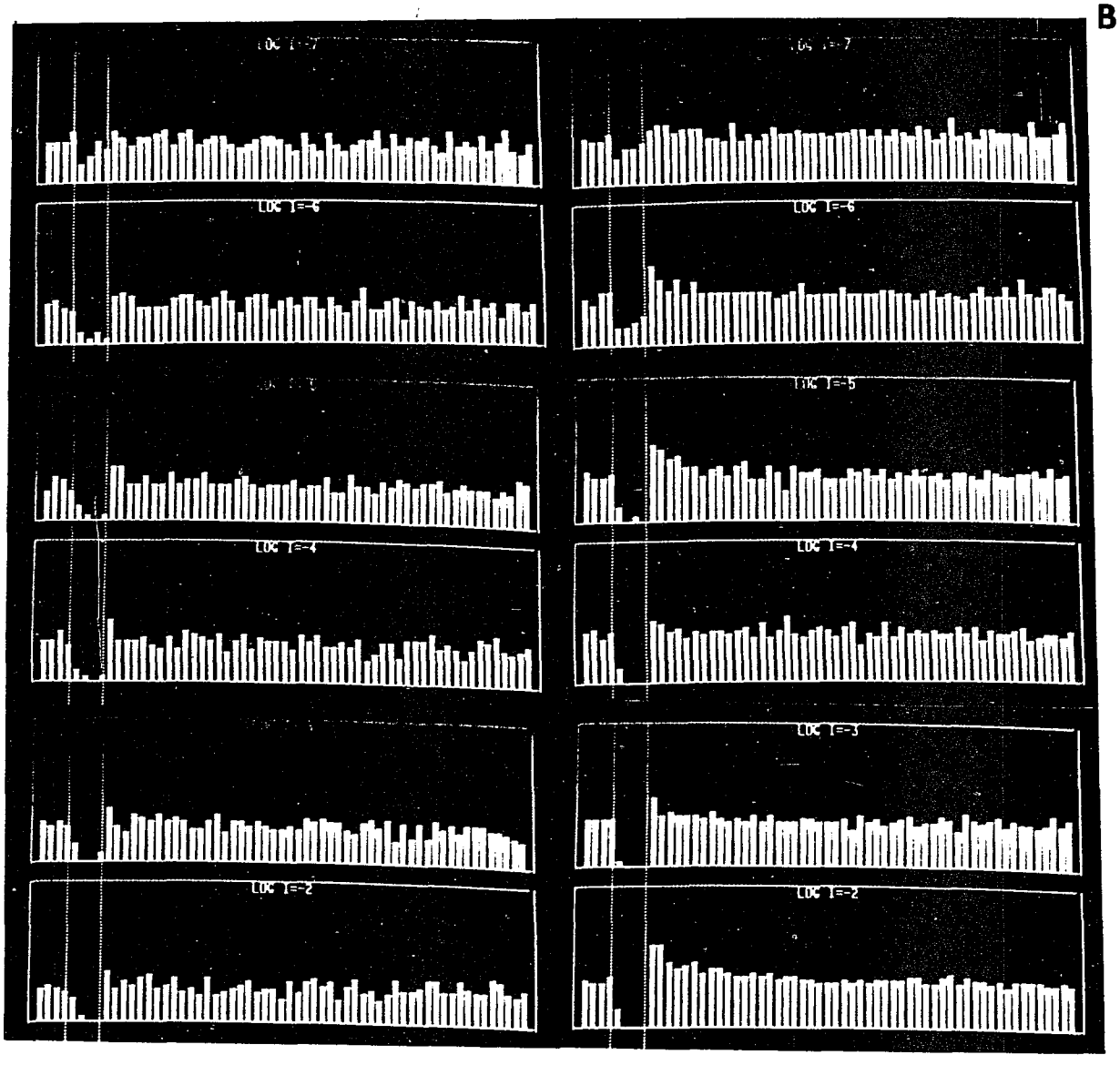



Figure 28

Figure 29

Interflash spike frequency.

A linear plot of the relationship of the interflash spike frequency, measured 30 seconds after light off, to the dose of curare. The curves represent the mean values obtained from 7 preparations. Interflash spike frequency was determined by averaging the four half second intervals prior to the second and third light stimulus (see methods: pharmacology, curare)(see figures 23-26) and then multiplying by 2 to convert the number to Hertz. The dark frequency (solid circle) was measured after the preparation was dark adapted for one hour at each drug concentration. Ten consecutive half second intervals were averaged and the resulting numbers were multiplyed by two to convert the value to Hertz.

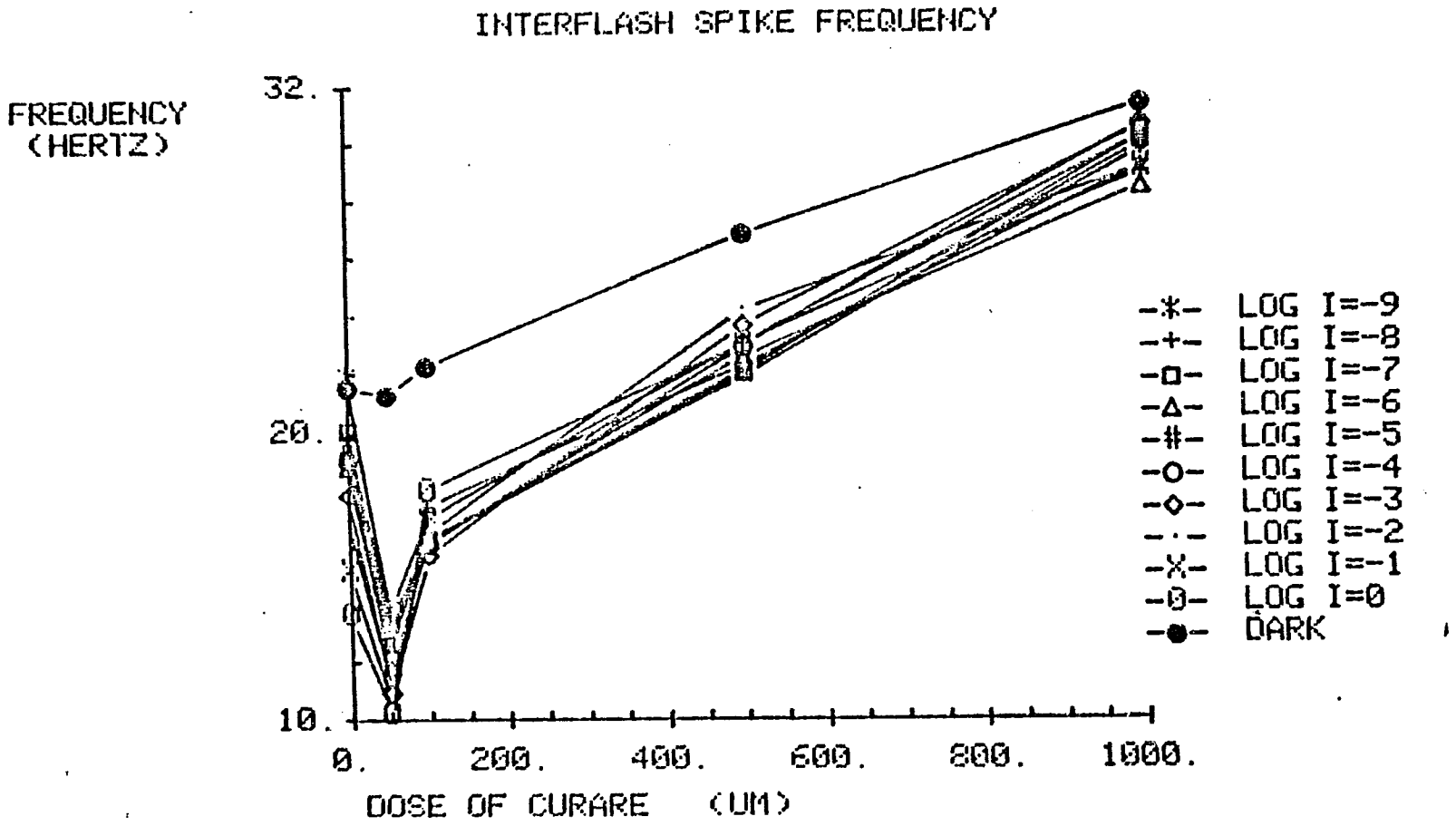

INTERFLASH SPIKE FREQUENCY
FREQUENCY
(HERTZ)
32.
20.
10.
0.
200.
400.
600.
800.
1000.
DOSE OF CURARE (UM)
LOG I=-9
LOG I=-8
LOG I=-7
LOG I=-6
LOG I=-5
LOG I=-4
LOG I=-3
LOG I=-2
LOG I=-1
LOG I=0
DARK

Figure 29

Figure 30

Interflash spike frequency

An alternative plot of the same data as presented in figure 29 showing the relatiosnship of the interflash spike frequency to the log intensity of the light flash at different concentrations of curare. It shows in another way that for the entire range of light intensities used, the interflash spike frequency in 50 uM curare is less than that of the control. A test of the slopes of the curves for Ringer and 50 um curare show that the slopes are different at $P<0.01$. A test of the independence of y vs x for the three highest drug doses show that the interflash spike frequency was independent of the light intensity ($P>0.05$), when the animal was perfused with curare at those concentrations.

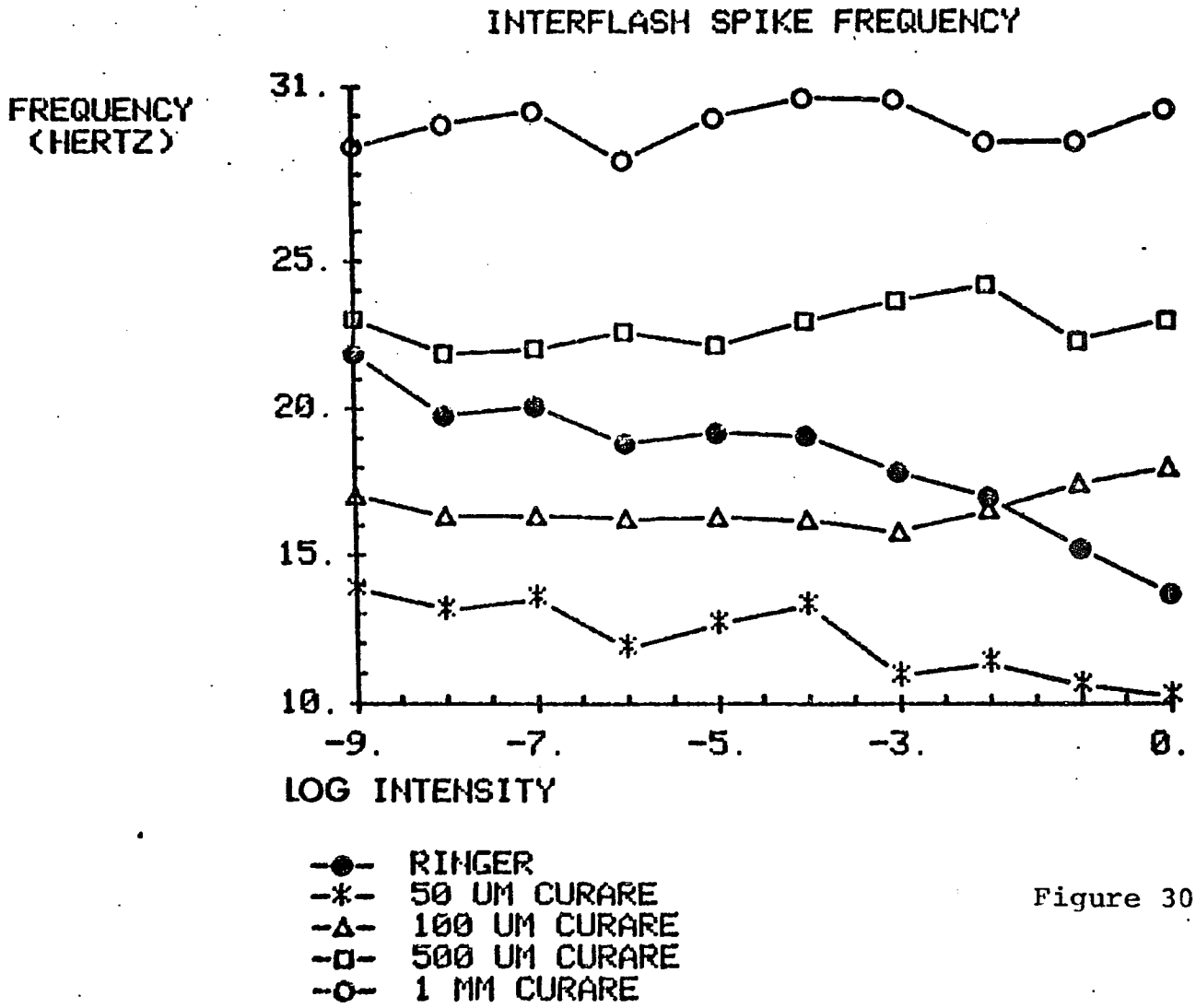

INTERFLASH SPIKE FREQUENCY
FREQUENCY (HERTZ)
31.
25.
20.
15.
10.
-9.
-7.
-5.
-3.
0.
LOG INTENSITY
RINGER
50 UM CURARE
100 UM CURARE
500 UM CURARE
1 MM CURARE

Figure 30

Figure 31

Average frequency in the light.

Intensity-spike frequency relationship in the presence of increasing concentrations of curare. The data was obtained by averaging the last 3 half second intervals of the second and third light flash. Each flash lasted for two seconds (see methods: pharmacology, curare). The average number of counts per interval was then multiplied by two to convert the number to Hertz. These curves represent data pooled from 7 preparations.

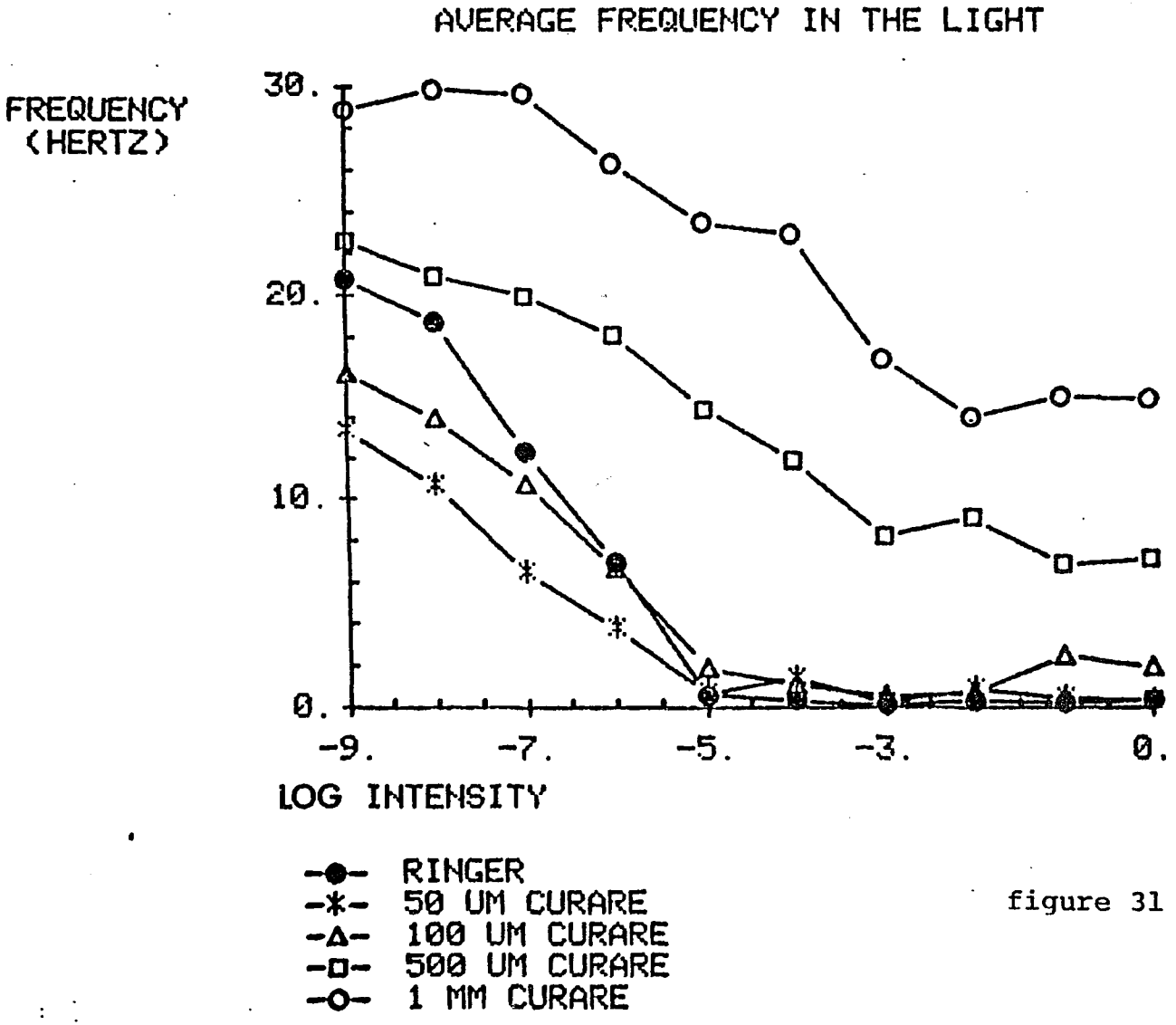

AVERAGE FREQUENCY IN THE LIGHT
FREQUENCY
(HERTZ)
30.
20.
10.
0.
-9.
-7.
-5.
-3.
0.
LOG INTENSITY
RINGER
50 UM CURARE
100 UM CURARE
500 UM CURARE
1 MM CURARE

figure 31

Figure 32

Statistical analysis of the light frequency.

Regression lines drawn through the points obtained from figure 31. A statistical test showed that the slope of the frequency vs intensity curve for Ringer is different from the slopes of the same curves obtained in the presence of the drug at $p<0.01$. All of the curves obtained in the presence of curare at all concentrations had identical slopes at $P>0.05$.

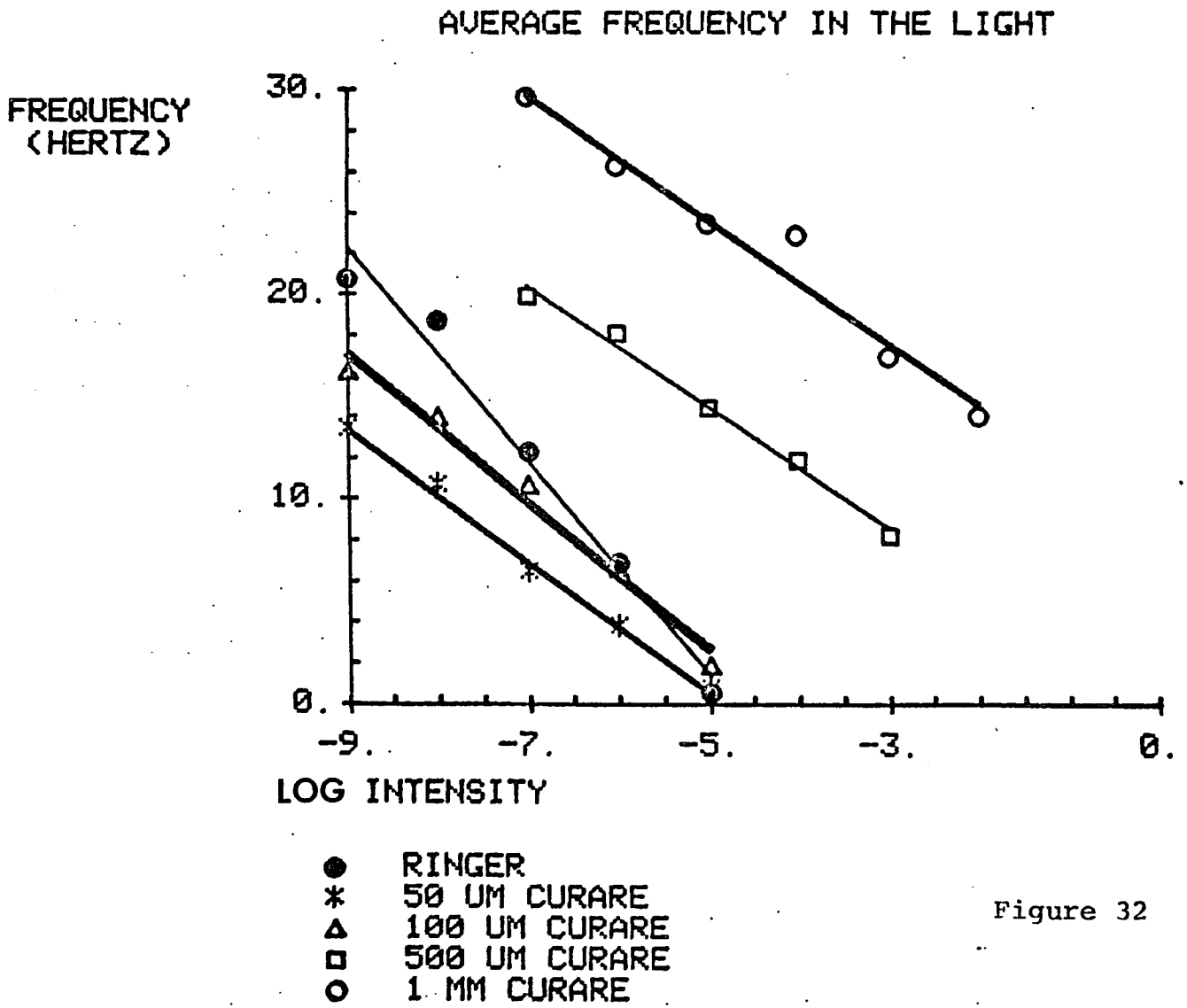

AVERAGE FREQUENCY IN THE LIGHT
FREQUENCY
(HERTZ)
30.
20.
10.
0.
-9.
-7.
-5.
-3.
0.
LOG INTENSITY
RINGER
50 UM CURARE
100 UM CURARE
500 UM CURARE
1 MM CURARE

Figure 32

Figure 33

Change in impulse firing rate

A graph showing the relationship between the change in spike frequency due to light vs the log intensity of the light at increasing concentrations of curare. The parameters were obtained by finding the average impulse frequency in the light as explained in figure 31 and subtracting it from the average frequency just prior to light "on" (the latter is the same as the interflash spike frequency obtained in figures 29 and 30).

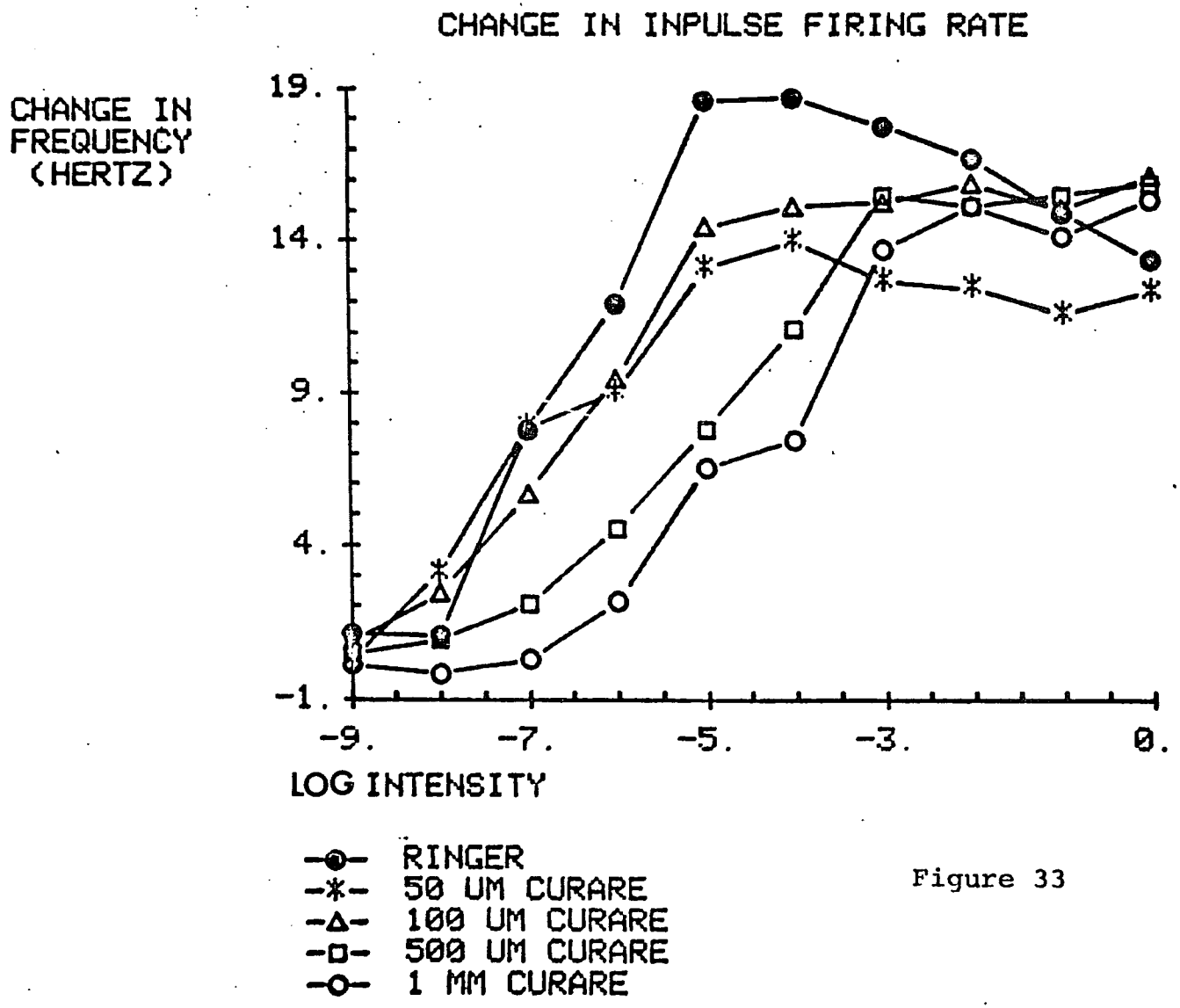

CHANGE IN INPULSE FIRING RATE
CHANGE IN FREQUENCY (HERTZ)
19.
14.
9.
4.
-1.
-9.
-7.
-5.
-3.
0.
LOG INTENSITY
RINGER
50 UM CURARE
100 UM CURARE
500 UM CURARE
1 MM CURARE

Figure 33

Figure 34

A statistical analysis of the change in spike frequency.

Regression lines drawn throught the points obtained from figure 33. A statistical test to determine if the slope of the curve for Ringer is identical to the slopes of the drug curves showed them not to be identical at $P<0.01$. All of the curves obtained in the presence of curare had identical slopes at $P>0.05$.

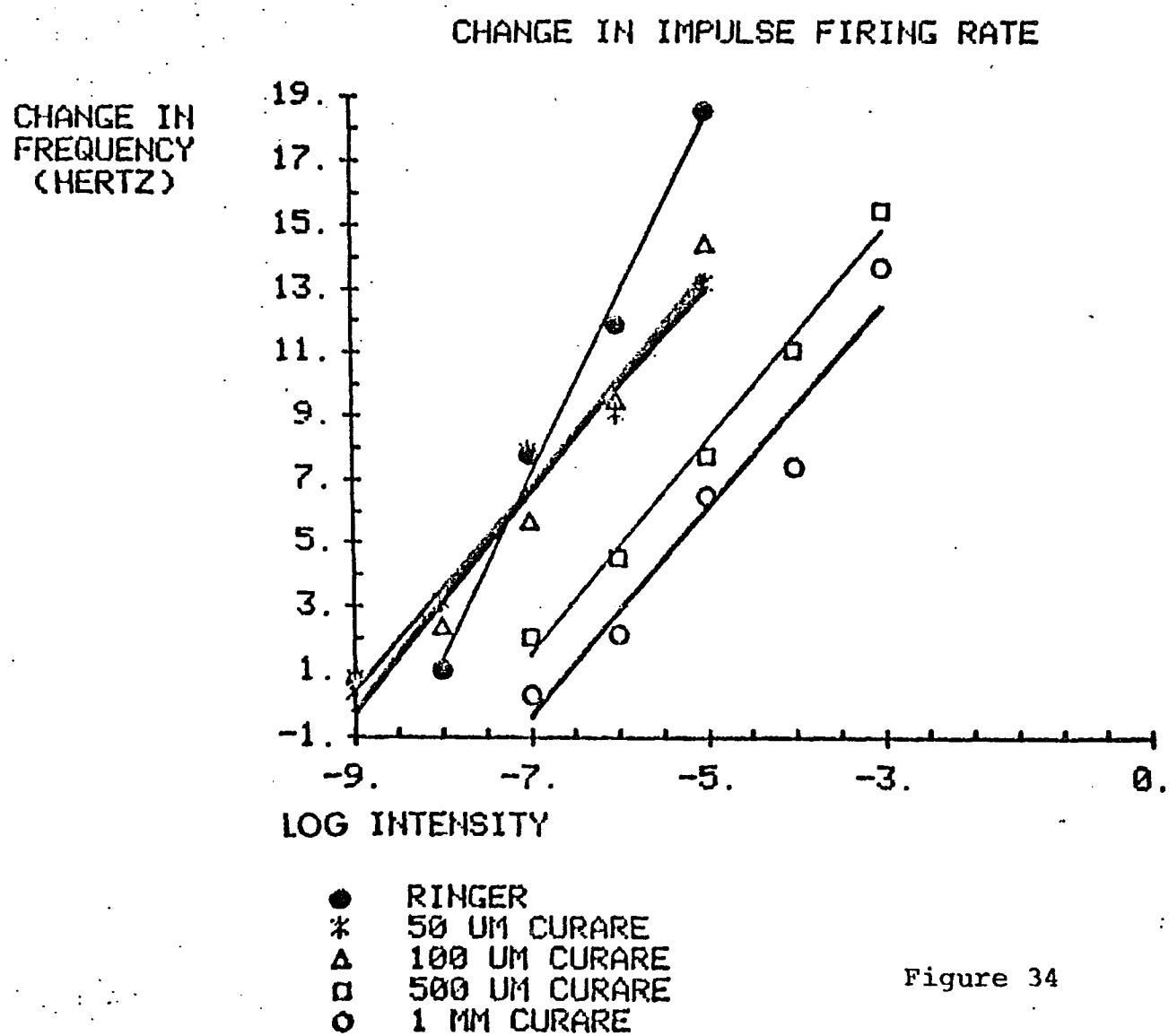

CHANGE IN IMPULSE FIRING RATE
CHANGE IN FREQUENCY (HERTZ)
19.
17.
15.
13.
11.
9.
7.
5.
3.
1.
-1.
-9.
-7.
-5.
-3.
0.
LOG INTENSITY
RINGER
50 UM CURARE
100 UM CURARE
500 UM CURARE
1 MM CURARE

Figure 34

Figure 35

Time course of the effects of GABA on impulse activity.

Each histogram represents the spike counts of three consecutive flashes. The preparation was stimulated every 18 seconds. Each bar represents an interval of 200 milliseconds. The time of illumination is denoted by the vertical bars. The concentration of GABA in the perfusate was 10 mMolar. Stimulus intensity: log I=-5.

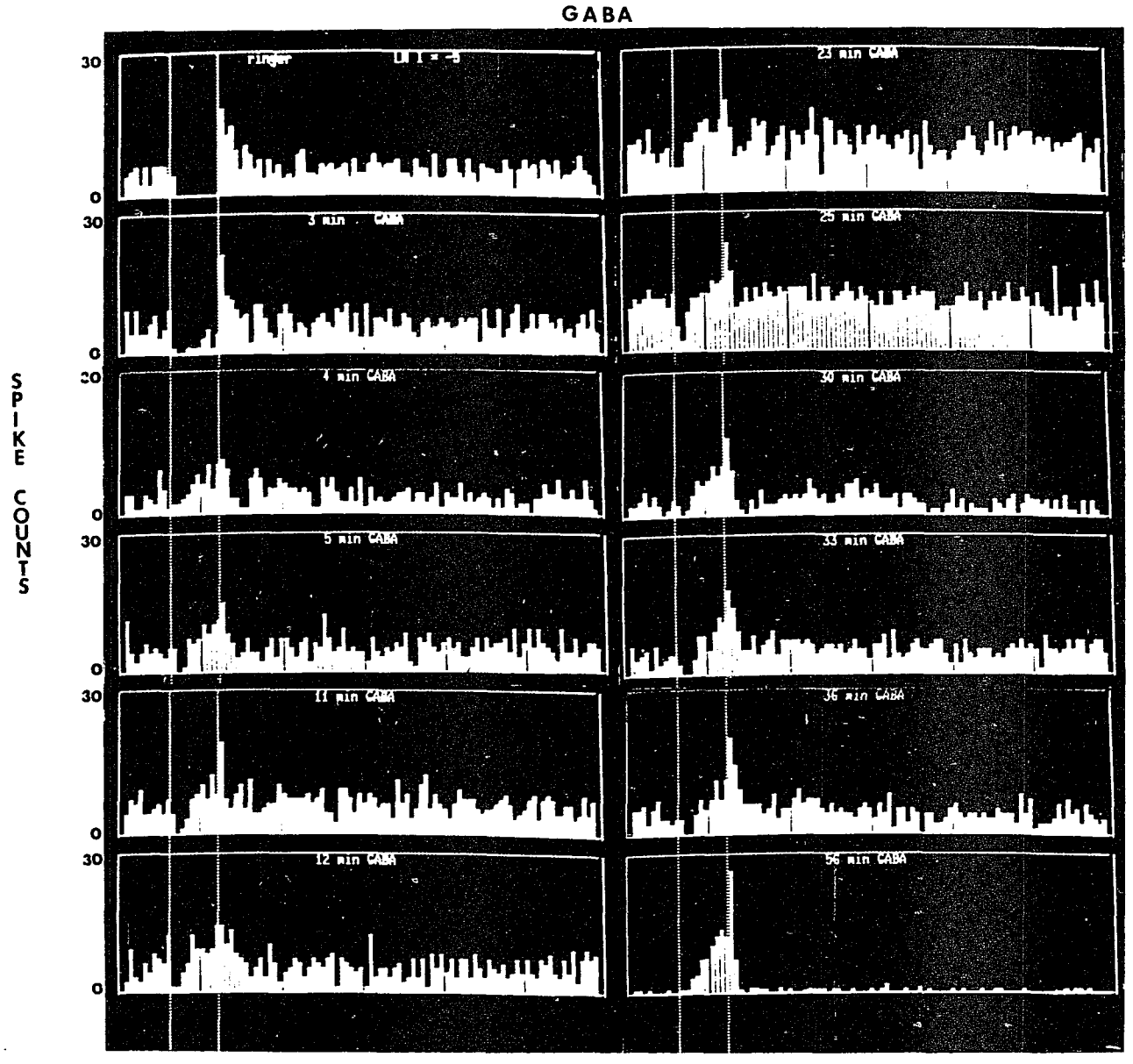

GABA
SPIKE COUNTS
30
0
3 min CABA
23 min CABA
25 min CABA
4 min CABA
30 min CABA
5 min CABA
33 min CABA
11 min CABA
36 min CABA
12 min CABA
56 min CABA

Fig. 35

Figure 36

Intensity-reponse series in the presence of GABA.

An intensity-response series comparing the behavior of the impulse activity in Ringer (upper histogram) and after over 60 minutes in 10 mM GABA (lower histogram) under increasing intensities of light. Time of illumination (2 seconds) is denoted by the vertical lines. Each histogram is the sum of the impulses at every interval for a total of six consecutive flashes. Each flash was two seconds long and occurred at a frequency of one every 18 seconds. Note LN = LOG10. A, dark. B, Log I=-8. C, Log I=-7. D, Log I=-6. E, Log I=-5. F, Log I=-4. G, Log I=-3. H, Log I=-2. I, Log I=-1.

Fig. 36

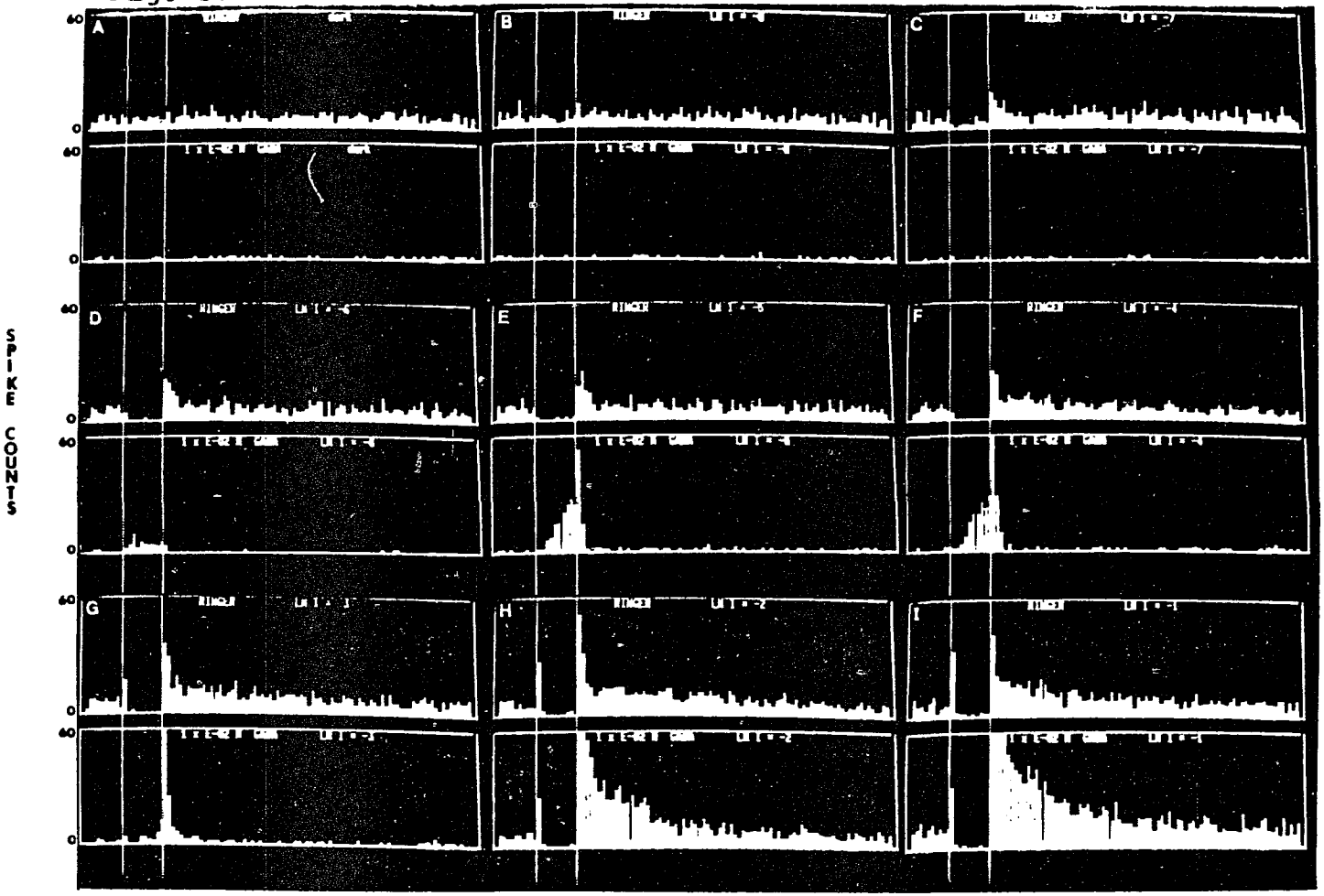


Figure 37

Light faciliation of impulse activity in the presence of GABA.

An intensity-response series in the presence of 10 mM GABA for one hour. As in the previous figure, Each histogram represents the sum of six flashes. The figure shows the shift of the light facilitation of the spikes from light "on" to light "off". Time of illumination (2 seconds) is indicated by the vertical bars. Note: LN = LOG10.

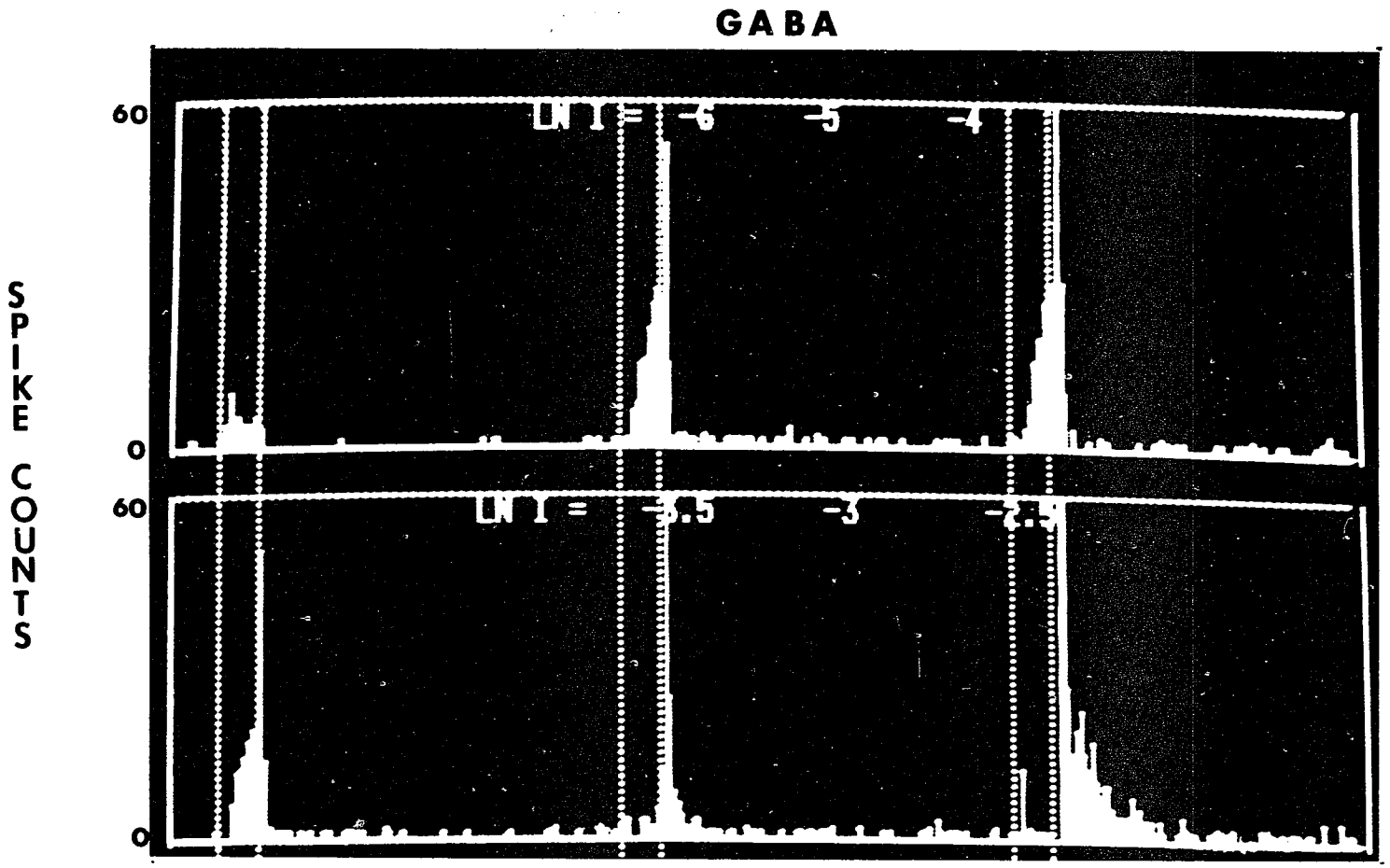



Figure 37

Figure 38

Time course of washing after a GABA perfusion.

The time course of washing the preparation after it had been perfused in 10 mM GABA for 90 minutes. Each histogram represents the sum of three consecutive flashes. Time of illumination (2 seconds)is denoted by the dark bars. Stimulus intensity: log I= -5.

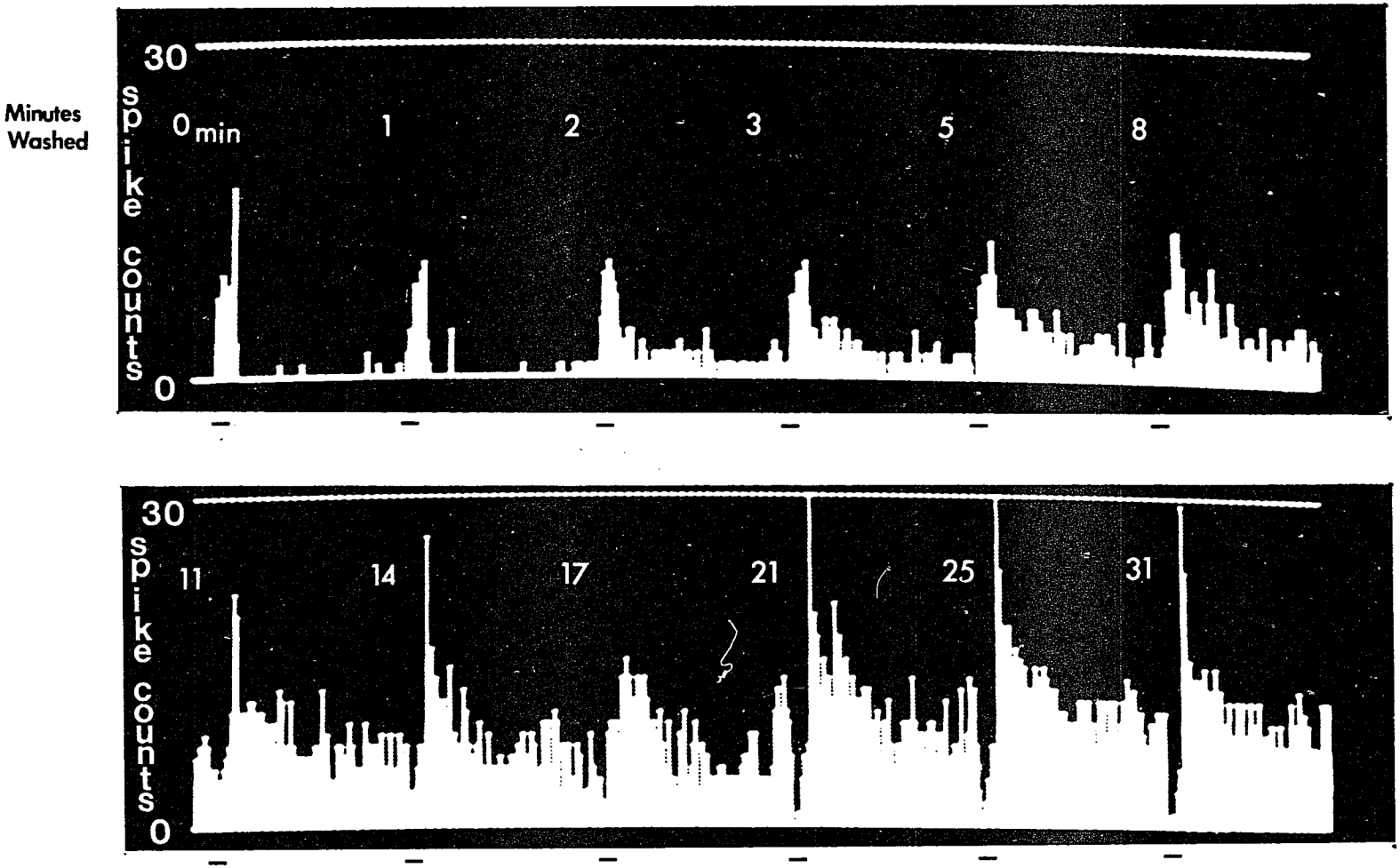

Minutes Washed
30
spike counts
0
0 min
1
2
3
5
8
30
spike counts
0
11
14
17
21
25
31

Figure 38

Figure 39

Time course of the action of cobalt.

12 mM cobalt reversibly inhibits ocellar nerve impulse activity after 18 minutes. Each histogram represents the total spike counts at each interval for 9 consecutive light flashes. The time of illumination (2 seconds) is denoted by the bar. Stimulus intensity is log I=-5.

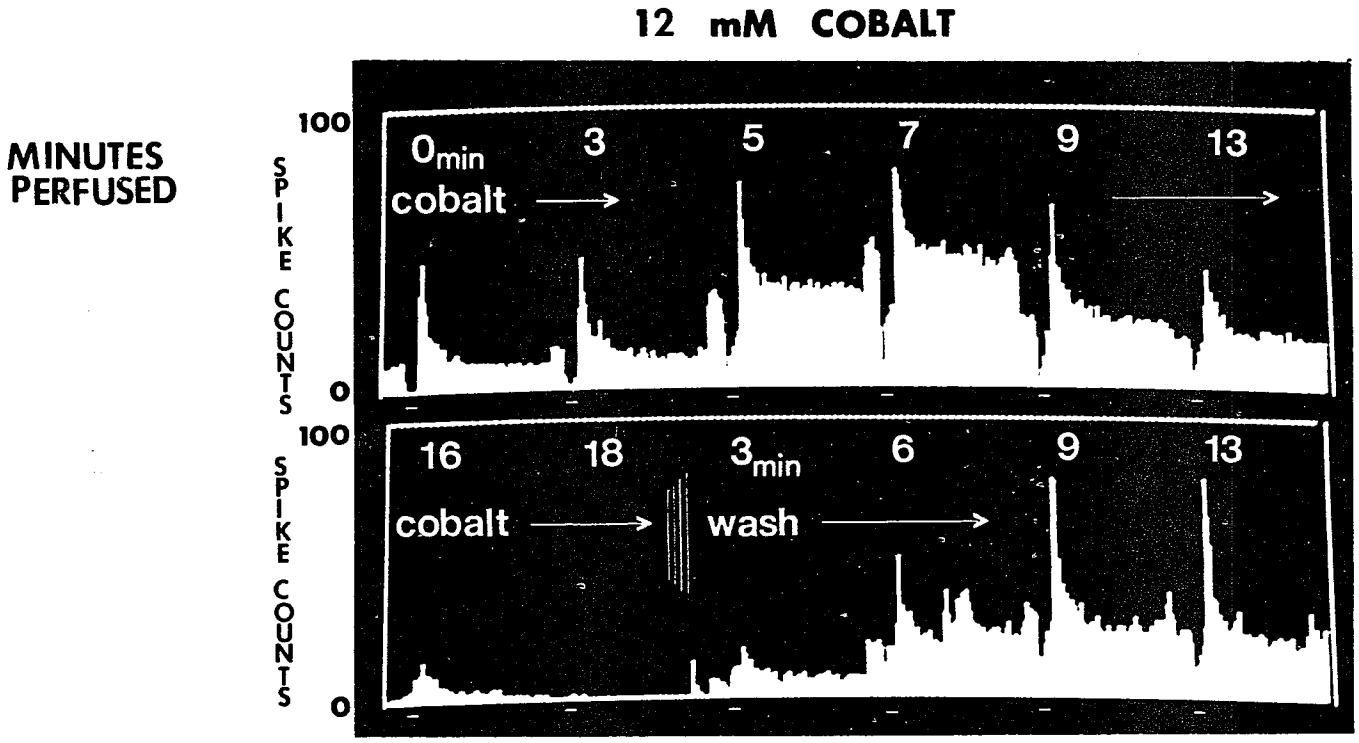

12 mM COBALT
MINUTES PERFUSED
100
SPIKE COUNTS
0
0min
cobalt
3
5
7
9
13
100
SPIKE COUNTS
0
16
18
3min
cobalt
wash
6
9
13

Figure 39

Figure 40

Changes in ocellar nerve impulse activity during a dim flash

This frequency histogram of ocellar nerve impulse activity was constructed using the "multispikes" program (see: Methods, "experimental protocol for pharmacological experiments...GABA and Cobalt"), by summing the spikes in each time interval for 30 consecutive two second flashes. The time between each flash was 22 seconds. Each time interval represents 0.2 seconds. The horizontal bar denotes time of illumination.

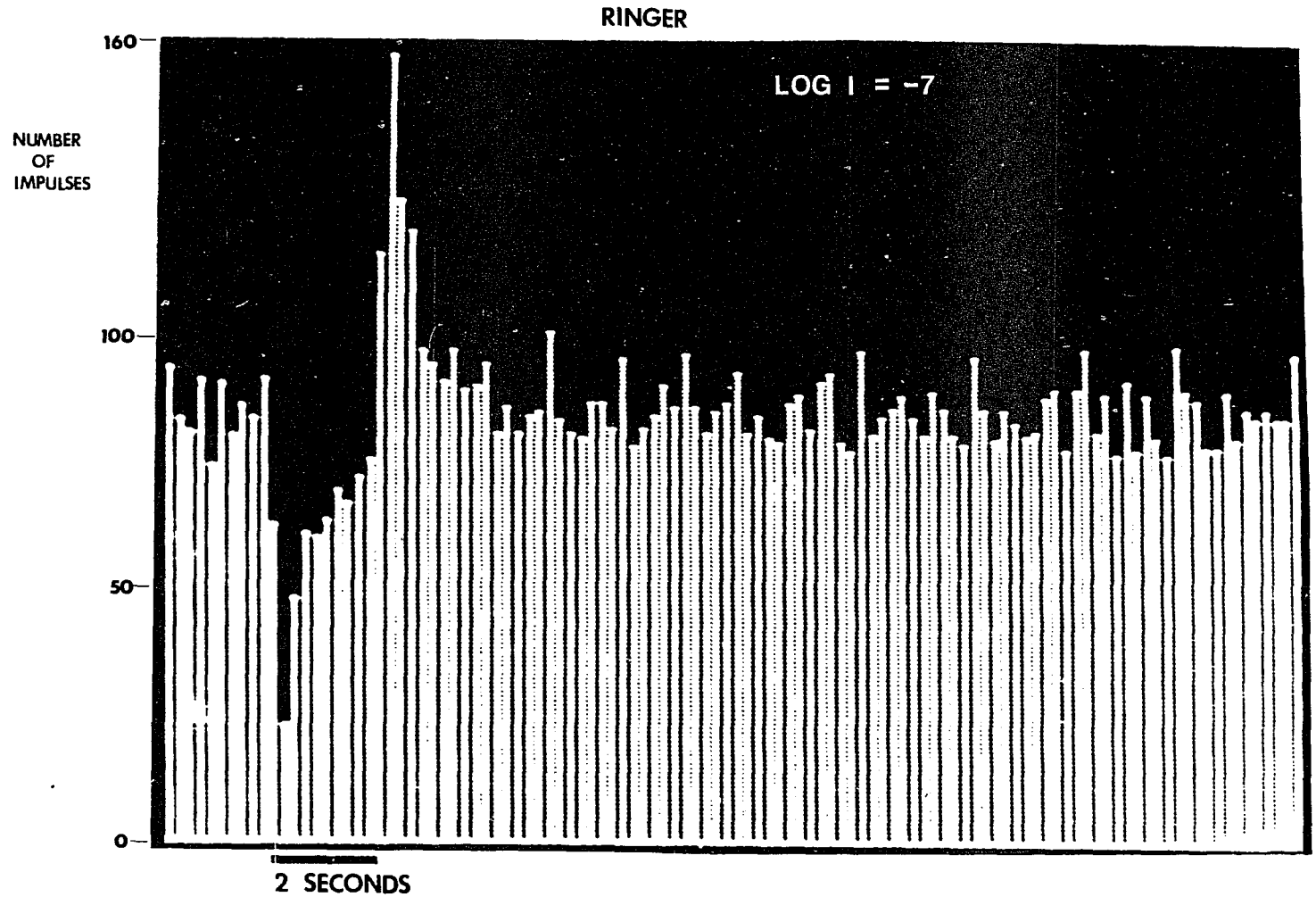



Figure 40

Figure 41

Dual threshold model

A and B represent the effective membrane potential at the site of spike initiation in the spiking neuron in the absence of any pharmacological agents. C and D represent this potential in the presence of GABA. A′,B′,C′, and D′ correspond to the patterns of impulse activity that the model predicts would be seen in the extracellular trace under the conditions described by the figure. The horizontal bars denote the time of illumination.

The effect of GABA is shown to maintain the membrane potential above the upper threshold when the preparation is stimulated with low levels of illumination. At higher intensities of illumination, the membrane potential, when the preparation is perfused with GABA (D), is still higher than that of the control (B) before and after the light stimulus. See text for further explanation.

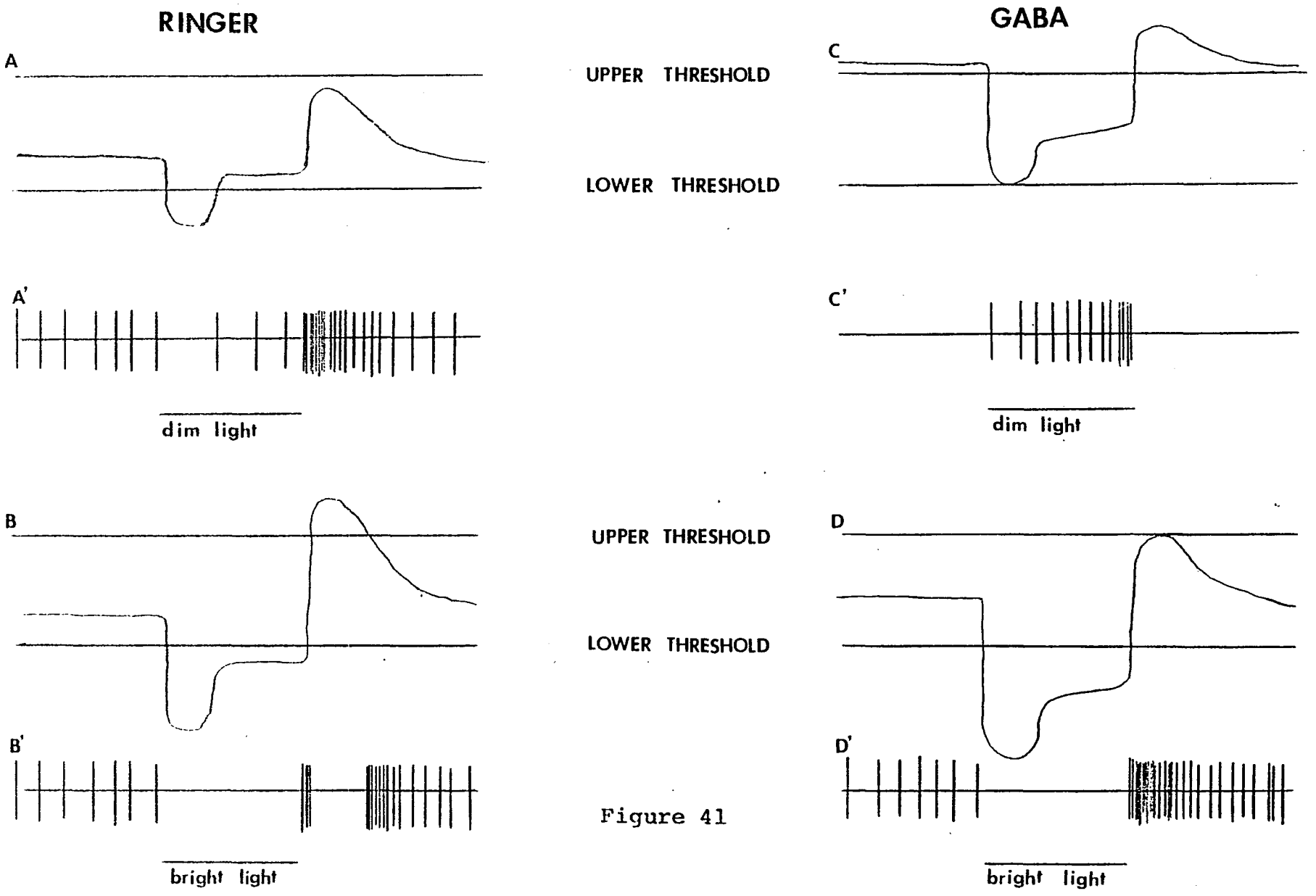

RINGER
GABA
A
C
UPPER THRESHOLD
LOWER THRESHOLD
A'
C'
dim light
dim light
B
D
UPPER THRESHOLD
LOWER THRESHOLD
B'
D'
bright light
bright light

Figure 41