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ELECTROPHYSIOLOGICAL ANALYSIS OF SYNAPTIC TRANSMISSION IN THE RETINA OF THE DRAGONFLY OCELLUS

by

ARLENE KLINGMAN

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

October 10, 1975
Date

Chairman of Examining Committee
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ABSTRACT

The response of the ocellar nerve dendrite, the second order neuron in the retina of the dragonfly ocellus, has been analyzed by means of intracellular recording and a model constructed to explain the generation of that response. Responses were recorded from ocellar nerve dendrites in 5 different species. The typical light response, similar in all species, consisted of a hyperpolarization with a large transient and a smaller sustained component, followed by an overshoot of the resting potential and oscillations after light-off. The hyperpolarization was accompanied by a resistance decrease. The transient nature of the ocellar nerve dendrite response compared with the receptor response, and anatomical evidence of synapses from the ocellar nerve dendrites back to the receptor terminals, led to the hypothesis that the ocellar nerve dendrite response reflected an interaction between synaptic input from the receptors and synaptic feedback from the ocellar nerve dendrites to the receptor terminals.

A method was devised for perfusing the ocellar nerve dendrite preparation with various solutions during intracellular recording, and it was found that the response could be modified by drugs. Curare blocked the response completely, while acetylcholine hyperpolarized the ocellar nerve dendrite.

Both picrotoxin and bicuculline eliminated the "off" overshoot, and bicuculline also decreased the size of the response and the sensitivity. GABA increased the size of the response.

The evidence suggests that the receptor transmitter may be acetylcholine and that the ocellar nerve dendrite feedback transmitter may be GABA. A model was developed to explain the typical ocellar nerve dendrite response and the effect of drugs on that response. According to the model, both receptor and feedback transmitters are released in the dark. Light increases the amount of receptor transmitter released. The receptor transmitter hyperpolarizes and inhibits the ocellar nerve dendrite and its feedback transmitter release. The feedback transmitter has a facilitory effect on the release of transmitter from receptor terminals.

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INTRODUCTION

In the last decade, intracellular recording and electron microscopy have made possible great advances in understanding the structure and function of the retina. From electrophysiological and anatomical studies in a variety of vertebrate and invertebrate retinas, certain common properties have begun to emerge. Among the most dramatic is the enormous amount of information processing which takes place in the retina. While individual receptor cells respond to light with graded, sustained potential changes, the output of the retina is more complex. Early studies of receptive field organization of frog (Hartline 1938, 1940) and cat (Granit, 1950, Kuffler, 1953) optic nerve fibres revealed a concentric arrangement of excitatory and inhibitory fields and transient bursts of impulses at "on," "off," or both. A higher degree of information processing was demonstrated in the frog retina by Maturana, Lettvin, McCulloch, and Pitts (1960) by using patterned stimuli rather than spots of light and moving them in various ways. The transformation of information across the retina from the receptors to the ganglion cells whose axons form the optic nerve was described by Werblin and Dowling (1969) who recorded from each type of neuron in the retina of *Necturus*, using spot and annular stimulation. They found that the amacrine cells

were the first cell type which gave a primarily transient response to illumination, and they point out the possible role of the reciprocal synapses of the amacrine cell process back onto the bipolar cells in converting the sustained response of the bipolar cell into the transient response of the amacrine cell. They suggest that this could be accomplished by the amacrine cell synapses turning off the bipolar excitation locally. A similar conversion from sustained to transient response occurs at the bipolar-amacrine level in goldfish (Kaneko, 1970) and carp (Toyoda, Hashimoto and Ohtsu, 1973).

The concept of synaptic feedback giving rise to transient responses seems to have general applicability in both invertebrate and vertebrate retinas. Ratliff, Hartline and Miller (1963) suggest such a role for lateral and self inhibition in the *Limulus* eye. Toyoda, Hashimoto and Ohtsu (1973) also suggest that in the carp, as in *Necturus* (Werblin and Dowling, 1969), the more transient nature of the amacrine cell responses compared with bipolar cell responses is probably due to the amacrine-to-bipolar feedback synapses. Dowling and Chappell (1972) and Chappell and Dowling (1972) found anatomical and electrophysiological evidence for synaptic feedback in the dragonfly ocellus. In this retina there are reciprocal synaptic arrangements between the

receptors and second order cells and both receptors and ocellar nerve dendrites make button synapses with post-synaptic dyads, similar to the ribbon synapses of vertebrate bipolar cells. The receptors give a sustained response to light, while the second order cell, which has its cell body in the brain, gives a much more transient response with an "off" overshoot associated with a burst of impulses. The present study is an attempt to dissect pharmacologically the straight-through and feedback synapses of the retina of the dragonfly ocellus, in order to investigate their relative contributions to the generation of the response of the second order ocellar nerve cell.

The dragonfly ocellus is a preparation well suited to a study of synaptic transmission in a retina. The retina of this simple eye has two types of neurons, receptor cells and ocellar nerve dendrites which go to the brain. The electrical activity of these two types of cells was first analyzed by Ruck (1961). From extracellular recordings he concluded that light stimulation induces a slow depolarizing potential in the receptor cells which results in a secondary depolarizing activity in the receptor cell axons. This leads to a hyperpolarizing potential in the dendrites of the ocellar nerve cells which inhibits their spontaneous activity. Ruck's analysis was confirmed by Chappell and Dowling

(1972) who recorded intracellularly from receptor cells and from ocellar nerve dendrites. In the receptors they recorded a graded depolarizing potential and a single tetrodotoxin sensitive impulse at light-on. In the ocellar nerve dendrite they recorded a hyperpolarizing response to light which has a higher transient-to-sustained ratio than the depolarizing response of the receptor cell. The ocellar nerve dendrite response has an overshoot of the resting potential at light-off followed by variably damped oscillations before returning to the resting potential. The ocellar nerve cells fire impulses in the dark which are readily recorded extracellularly but are rarely seen on intracellular records. These impulses are inhibited during illumination, at which time intracellular recordings show hyperpolarization. On extracellular records there is a high frequency burst of impulses at light-off before the discharge returns to the dark adapted frequency. This burst coincides temporally with the overshoot of the dark potential recorded intracellularly. When impulse activity is blocked by tetrodotoxin, the intracellularly recorded ocellar nerve dendrite response is unchanged, indicating that slow potentials are sufficient for synaptic transmission (Chappell and Dowling, 1972).

The intracellular ocellar nerve dendrite response of

the dragonfly ocellus is similar to the intracellular response of the second order monopolar neuron recorded in the compound eyes of the blowfly Calliphora (Zettler and Jarvilheto, 1971) and of the dragonfly (Laughlin, 1973).

In summary, the conversion from a sustained to a transient response, the importance of slow potentials in synaptic transmission, the existence of complex synaptic structures involving a presynaptic organelle and a postsynaptic dyad and reciprocal synaptic relationships between cells, combined with its relative simplicity, make the dragonfly ocellus a useful preparation in which to attempt to elucidate what appears to be a general property of retinas: the generation of transient responses by means of reciprocal synaptic interactions. Before the present study was begun, only a small number of intracellular ocellar nerve dendrite responses had been recorded (Chappell, 1970; Chappell and Dowling, 1972). My goal in this study was to record the response to a light stimulus from a large population of ocellar nerve dendrites in order to more definitively characterize it, and to attempt to analyze the synaptic process involved in its generation. The initial hypothesis, based on previous data (Chappell and Dowling, 1972; Dowling and Chappell, 1972) was that the ocellar response reflected an interaction between synaptic input from the receptors and

synaptic feedback from the ocellar nerve dendrites to the receptor terminals.

The button and conventional synapses of the dragonfly ocellus have the features generally associated with chemical transmission: clusters of vesicles on one side (presumably presynaptic) of the synapse, the synaptic cleft separating the two membranes, and thickening of the membranes on both the pre- and postsynaptic sides of the synapse (Eccles, 1964). This raised the possibility that transmission at these synapses could be blocked with drugs, and in the event that different transmitters were released by the receptor terminals and the ocellar nerve dendrites, the two types of synapses could be dissected pharmacologically.

Although no retinal neurotransmitters have yet been definitively identified, the most intensive research to date has involved vertebrate retinas. The approach has generally been to investigate the effect on the retina of various substances believed to be neurotransmitters or antagonists. Several amino acids were found to have striking effects. Glutamate and aspartate depolarize horizontal cells and block S potentials (Cervetto and MacNichol, 1972; Murakami, Ohtsu and Otsuka, 1972). Gamma-aminobutyric acid (GABA) inhibits the firing of retinal ganglion cells in rabbit (Noell, 1959) and cat (Straschill and Perwein, 1969).

GABA also hyperpolarizes and inhibits light-evoked impulses in the eccentric cell of the eye of Limulus (Adolph, 1966). In the frog retina picrotoxin, a GABA antagonist, was found to increase the "on" component of the proximal negative response, an extracellular potential which is thought to reflect amacrine cell activity (Burkhardt, 1972). This finding is consistent with the localization of GABA in amacrine cells in frog (Graham, 1972) and rabbit (Kuriyama, Sisken, Haber and Roberts, 1968), and the possibility that inhibitory synapses from amacrine cells to bipolar cells reduce the excitatory input from bipolars to amacrines (Werblin and Dowling, 1969). Picrotoxin was also found to reduce inhibition in Limulus (Adolph, 1966).

Although the most striking results have been obtained with amino acids, there are some reports of cholinergic effects in vertebrate retinas. Ames and Pollen (1969) found that ganglion cell firing was increased by anticholinesterases and nicotine (a cholinomimetic), and decreased by d-tubocurarine and atropine (cholinergic antagonists). Galib and Liebman (1974) have reported that in the frog retina darkness, which depolarizes the photoreceptors, induces release of acetylcholine and that this release is reduced in the light when the receptors are more hyperpolarized. However, in fish retinas, acetylcholine was found to have no effect on receptors or horizontal cells (Murakami, Ohtsu

and Ohtsuka, 1972). Catecholamines were also found to effect activity in vertebrate retinas. Dopamine and norepinephrine inhibited activity of ganglion cells in rabbits (Ames and Pollen, 1969) and cat (Straschill and Perwein, 1969).

In view of the sensitivity of retinal neurons to a wide variety of substances, it seemed that it might be possible in the dragonfly ocellus, through an empirical approach, to interfere separately with receptor-to-ocellar nerve dendrite transmission and with the feedback transmission, and from this to try to determine how these two components contribute to the normal ocellar nerve dendrite response.

As a secondary approach to an analysis of the ocellar nerve dendrite response, I hoped to explore the possibility of developing a receptor preparation free of synapses with ocellar nerve dendrites by cutting the ocellar nerve during development. Such a preparation was developed in the housefly _Musca domestica_ by Eichenbaum and Goldsmith (1968) by transplanting the compound eye imaginal disc to the abdomen in the larval stage. If a similar non-innervated receptor could be produced in the dragonfly, it might be possible to record receptor responses unmodified by feedback. However, this project was considered exploratory and secondary to the electrophysiological analysis of the

intracellular ocellar nerve dendrite response through pharmacological manipulation of that response.

METHODS

PREPARATION

Experiments were performed on dragonflies caught at Van Cortlandt Park in New York City and on dragonflies raised in the laboratory according to the procedure developed by Chappell (1970) from nymphs supplied by Connecticut Valley Biological Supply Co., Southampton, Mass. Nymphs were kept in aquarium tanks divided into individual compartments by perforated plastic dividers and wire screens in order to prevent cannibalism, and were fed daily on tubifex worms.

All records presented here were recorded from *Aeschna tuberculifera*, *Libellula pulchella*, *Plathemis lydia*, and *Erythemis simplicicollis*. *Anax junius* was also recorded from but not shown here. Identification of species was based on specimens previously identified by Dr. Oliver S. Flint, Supervisor and Curator of the Division of Neuropterids, Department of Entomology, the Smithsonian Institution, Washington D.C. (Chappell, 1970). Receptor and ocellar nerve dendrite responses were similar in all species used, whether wild or laboratory-raised.

DISSECTION

Experiments were performed on dragonfly heads which have been removed from the body and part of the exoskeleton

removed from the top of the head to expose the receptor structure and ocellar nerve in situ, according to the method of Chappell (1970) and Chappell and Dowling (1972).

PERFUSION

For the drug experiments which were to be attempted in this study it was necessary to be able to turn drug solutions on or off during intracellular recording without disturbing the preparation, to apply drugs continuously over long periods, and to be able to switch from one solution to another during recording. To accomplish this I designed and assembled the apparatus shown in figure 1. The dissected dragonfly head is mounted with Tackiwax on a small, obliquely cut cork which is fitted into a round depression in the floor of the plexiglass chamber. Mounted on a stand above the head are 3 inch long 30 gauge needles which are connected by PE 90 Intramedic tubing to 10 ml syringes fitted with Luer Lok valves which can be opened or closed during recording. When the valves are opened the solutions empty by small droplets at a rate of about 1 ml in 20 minutes. The needles are positioned so that when the valves are opened drops of Ringer solution with or without drugs fall into the head through the opening made in the exoskeleton. At the beginning of an experiment the head is placed at an appropriate level on the cork and Ringer solution is placed

in the plexiglass chamber so that it is just high enough to make contact with the bottom of the pool of Ringer which accumulates in the head. This pool drains continuously out through the bottom of the head into the chamber and out of the chamber via a cotton wick hung over the side, into a glass Petri dish which holds the chamber. The silver-silver chloride reference electrode screws into a threaded hole in one side of the chamber. This apparatus allows continuous perfusion with Ringer solution from one of the syringes during the time required to locate and penetrate a cell, and also permits rapid switching to perfusion with a drug solution by simply closing and opening the appropriate valves.

The Ringer solution used had the following composition: 134 mM NaCl, 5.4 mM KCl, 3.8mM $CaCl_2$, 3.0 mM $MgCl_2$, 0.5 mM $NaHCO_3$; pH 7.4. Ionic composition of the Ringer was based on an analysis of *Aeschna* nymph hemolymph by Duchateau, Florkin and Leclerq (1953).

Drugs were dissolved directly in the Ringer solution, except for bicuculline which was prepared by first dissolving bicuculline in a few milliliters of 0.1 N HCl, then adding Ringer, followed by drops of NaOH until the bicuculline just began to come out of solution, at about pH 6.3.

Drugs used, and their sources, were as follows: acetylcholine chloride, Pfaltz and Bauer, Inc., Flushing,

N.Y.; atropine sulfate, Mann Research Laboratories, New York, N.Y.; bicuculline, K&K Laboratories, Inc., Plainview, N.Y. and Pierce Chemicals, St. Louis, Mo.; d-tubocurarine chloride, Mann Research Laboratories, New York, N.Y.; edrophonium chloride, Hoffmann - La Roche, Inc., Nutley, N.J.; gamma-amino-n-butyric acid, Sigma Chemical Co., St. Louis, Mo.; picrotoxin, Sigma Chemical Co., St. Louis, Mo.

INTRACELLULAR RECORDING

Glass micropipette electrodes were made from Corning Type 7740 capillary tubing having 1 mm outside diameter and 0.5 mm inside diameter, using a David Kopf 700B Vertical Pipette Puller (David Kopf Instruments, Tujunga, Calif.) or a Narishige PD-5 Glass Micro-Electrode Puller (Narishige Scientific Instrument Laboratory, Tokyo). Glass fibers were inserted in the capillary tubing before pulling, and the electrodes were filled by injecting 2M KCl with a 30 guage needle after the method of Tasaki, Tsukaharo, Ito, Wayner and Yu (1968). Electrodes having tip resistances of 50 to 100 MΩ when measured in Ringer solution were used for recording.

The microelectrode holder (Bioelectric Instruments, Farmingdale, N.Y.) contained a chlorided silver wire in contact with Ringer solution which in turn contacted the KCl in the electrode. An identical chlorided silver wire im-

mersed in the solution bathing the preparation served as the reference electrode. Leads from the electrodes went to a Mentor N-950 Intracellular Probe System (Mentor Corporation, Minneapolis, Minn.). The d.c. potential between the two electrodes was displayed on a Tektronix Type 502 Dual Beam Oscilloscope and also recorded on magnetic tape by a four channel FM tape recorder (A.R. Vetter Co., Rebersburg, Pa.). Tapes were later played back onto a Tektronix type 564 Storage Oscilloscope for analysis of data.

RESISTANCE MEASUREMENTS

Membrane resistance changes during illumination were observed by passing trains of 10 or 20 msec, 1 nAmp depolarizing pulses every 100 msec through the Mentor N-950 bridge system. The bridge balance potentiometer was adjusted to give a null reading of pulse amplitude in the dark. Cell membrane resistance changes during illumination cause a displacement of the pulse-on portion of the oscilloscope trace. Figure 2 shows oscilloscope traces obtained with a microelectrode in Ringer solution. In the upper trace the bridge potentiometer was adjusted for a null reading. The downward transient marks the beginning of the pulse. In the middle trace the potentiometer has been set at a higher value. This gives a downward displacement of the pulse-on portion of the trace. A similar displacement would be seen

if the potentiometer setting had not been changed but the electrode resistance (or the cell membrane resistance when recording inside a cell) had decreased. The opposite situation is shown in the lower trace. The potentiometer setting has been turned down below the null level, thus giving an upward displacement equivalent to that which would be caused by an increase in membrane resistance.

PHOTOSTIMULATOR

A 100W Osram quartz-iodide bulb controlled by a 8.3 amp d.c. constant current source was used to illuminate the ocellus. The beam was brought to focus onto one end of a 36 inch, 3mm diameter fiber optic bundle which was positioned 5 mm from the median ocellar lens during an experiment. The intensity of light at the ocellar lens was calculated as 170 footcandles by the following method: the fiber optic bundle was placed directly against the footcandle diffuser attached to the photometric filter of an Optometer Model 40X light meter (United Detector Technology, Santa Monica, Calif.) traceable to the National Bureau of Standards. A reading of 56 footcandles was obtained. However, the Model 40X light meter is designed to give a direct footcandle reading only if the entire 1 cm^2 area of the photocell is illuminated, which was not the case here. The above method was useful in monitoring the constancy of the light source, but it also

seemed desirable to get an estimate of the actual intensity at the ocellus. In order to do this, the following calculation was made:

The distance from the end of the fiber optic to the lens of the ocellus was 5 mm. The diameter of the light spot at this distance was 3.25 mm, giving an area of .083 cm^2. The area of the photocell was 1 cm^2, so the reading of 56 footcandles was multiplied by 1/.083 to give a corrected value of about 675 footcandles. This intensity is referred to as log I = 0, with lower intensities (referred to as log I = -1, etc.) produced by using Kodak Wratten neutral density filters to attenuate the beam.

RESULTS

Dissections were performed and intracellular recording attempted on approximately 400 dragonflies. From among these, 45 ocellar nerve dendrites were penetrated by microelectrodes and held long enough to record responses to light. The 45 included the following species: 29 *Aeschna tuberculifera*, 9 *Plathemis lydia*, 3 *Anax junius*, 1 *Libellula pulchella*, 1 *Erythemis simplicicollis*, and 2 unidentified species. The low rate of successful recording was due to a number of difficulties. In order to place a microelectrode in an ocellar nerve dendrite, the electrode was first aimed visually at the lateral portion of the ocellar nerve just above the point where the right and left halves of the nerve come together. This is the area where the ocellar nerve processes appear to be largest, according to the micrographs by Chappell and Dowling (1972) and Dowling and Chappell (1972). Other parts of the ocellar nerve were also explored initially, but successful intracellular recordings were much less frequent outside the area described above. After placing the microelectrode in the proper position above the preparation, a hydraulic microdrive was used to advance it into the tissue until penetration of a cell was indicated by two signs: the appearance of an inside-negative resting potential, and the appearance of a hyperpolarizing response

to light. In the vast majority of preparations in which recording was attempted, either no ocellar nerve dendrites were penetrated or after penetration both the resting potential and the response to light degenerated rapidly.

Most of the 45 ocellar nerve dendrites from which I recorded had resting potentials between -35 and -55 mV, but units with resting potentials as low as -18 mV displayed responses similar to those in cells with larger resting potentials. In one unit, impulse activity was seen during intracellular recording. This is shown in figure 3. Chappell and Dowling (1972) similarly found that impulse activity could not be recorded inside most ocellar nerve dendrites.

The responses recorded in these 45 units were for the most part similar to those reported by Chappell (1970) and Chappell and Dowling (1972). In 30 ocellar nerve dendrites an intensity-response series was recorded from log I = -6 to log I = 0. A typical intensity-response series, recorded from Aeschna tuberculifera, is shown in figure 4. Threshold for most units was log I = -5, and the maximum amplitude of the "on" transient was obtained at log I = -3 or -2. The average maximum amplitude of the "on" transient was -22 mV, with 33% between -20 mV and -25 mV. Compared with the responses shown by Chappell and Dowling (1972), the "off"

responses recorded in this study were more oscillatory, with both overshoots and undershoots of the resting potential, while those shown by Chappell and Dowling (1972) appear to return more directly to the resting potential after a more prolonged depolarization. The various species used in this study all gave similar responses.

Although the object of this study was to analyze the ocellar nerve dendrite response, intracellular recordings were also made in a few receptors. An intensity-response series from one of these is shown in figure 5. With higher intensities there is a single spike at light-on and a slow return to the resting potential after light-off. Some receptors also showed the oscillations after light-off reported by Chappell and Dowling (1972).

ANALYSIS OF OCELLAR NERVE DENDRITE RESPONSE

RESISTANCE MEASUREMENTS

The resistance of the ocellar nerve dendrite membrane decreased during illumination, as shown in figure 6. The pulse-on portion of the trace is displaced downward during illumination, indicating that the membrane resistance has decreased. A similar resistance decrease during illumination was observed in the two other preparations in which resistance measurements were obtained.

MODIFICATION OF OCELLAR NERVE DENDRITE RESPONSE

After recording inside several ocellar nerve dendrites and observing the responses described above, I attempted to interfere in various ways with the process by which the ocellar nerve dendrite response is generated in the hope of learning something about that process. Surgical intervention during development as described in the Introduction, was attempted but found to be unfeasible and abandoned. For details see Appendix. At this point I decided to concentrate on modifying the ocellar nerve dendrite reponse by means of drugs. Before this, the only experiments of this nature in the dragonfly ocellus involved topical application of tetrodotoxin (Chappell, 1970; Chappell and Dowling, 1972).

In the drug experiments, in addition to the difficulties described above in penetrating and holding ocellar nerve dendrites, there were further problems. First, the time needed to record responses, apply a drug, record, wash with Ringer solution, and record responses again, greatly reduced the probability of holding a cell throughout an entire experiment. Second, although the valves which controlled the flow of solutions could be opened and closed without disturbing the microelectrode, the manipulations required to do this did in fact increase the likelihood of

accidentally displacing the electrode. Because of these difficulties, the probability of completing any particular experiment was low, but this was to a great extent overcome by attempting a large number of experiments.

By trying a number of drugs, I found that I was able to modify the ocellar nerve dendrite response in two different ways: (1) blocking the ocellar nerve dendrite response completely, and (2) eliminating the "off" overshoot. These two effects will be presented separately.

BLOCKING THE OCELLAR NERVE DENDRITE RESPONSE

The light response of the ocellar nerve dendrite was completely and reversibly blocked by 10^{-4} g/ml and 10^{-3} g/ml d-tubocurarine chloride (curare). Figure 7 shows the response of a unit before drugs, after 4 minutes perfusion with 10^{-3} g/ml curare, and after washing 30 minutes with Ringer's solution. Within the first 3 minutes after curare perfusion was begun, there was a 4 mV positive shift in the resting potential. A complete block of the light response was also achieved in another preparation with this concentration of curare (see figure 8), in this case with no change in the resting potential, but after switching to the Ringer's solution for washing, the cell was lost, as indicated by a rapid decline in the resting potential. In another preparation the ocellar nerve dendrite response was

reversibly blocked by 10^{-4} g/ml curare. This is shown in figure 9. The light response of this unit is somewhat atypical in that there is a notch in the initial hyperpolarization. This was also seen in a few other units. After about 15 minutes perfusion with 10^{-4} g/ml curare, the light response has essentially been eliminated, although a potential change is still recorded at light-on. It is not clear what this remaining potential represents, but it does not indicate irreversible damage to the cell, since the light response is restored, although somewhat smaller in amplitude, after washing with Ringer's solution. One possibility is that it represents electrical activity of the receptors, which according to Zettler and Jarvilheto (1971), can be recorded at certain locations in the second order monopolar neuron of the compound eye of _Calliphora_. Perfusion with a lower concentration of curare, 6×10^{-6} g/ml, was begun in 5 preparations, but no positive results were obtained. In two of the preparations, the cell was lost within a few minutes after curare perfusion was begun. In two other preparations, after about 10 minutes of perfusion with the drug the responses began to decrease in size, but in both cases the resting potential also decreased and the cells were lost, so the decrease in the light response cannot be attributed to the drug. In the fifth preparation,

the light response was not reduced after 20 minutes perfusion with 6 x 10^{-6} g/ml curare.

One ocellar nerve dendrite preparation was perfused for 25 minutes with 10^{-4} g/ml atropine sulfate. The size of the response was somewhat reduced, as shown in figure 10, but there was no blocking effect comparable to that of curare.

BLOCKING THE "OFF" OVERSHOOT

The "off" overshoot was found to be the most drug-sensitive portion of the ocellar nerve dendrite response. It was altered or eliminated by both picrotoxin and bicuculline.

PICROTOXIN

The "off" overshoot was completely eliminated in 2 preparations by 10^{-3} g/ml picrotoxin. Figure 11 shows intensity-response series recorded from one of these preparations, before and after picrotoxin. The effect of 10^{-3} g/ml picrotoxin on the other preparation was the same. In both units, the "off" response changed in a characteristic way before disappearing. This change, in which the depolarized phase is greatly increased, is shown in figure 12. In both units, washing with Ringer's solution only restored the "off" response to this intermediate state, as shown in figure 13.

The "off" overshoot was also sensitive to lower concentrations of picrotoxin. In two preparations I was able to perfuse with 10^{-4} g/ml picrotoxin long enough to see a change in the "off" response. This change was similar to the "off" response seen briefly when beginning perfusion with 10^{-3} g/ml and also after washing out 10^{-3}g/ml, and, as noted above, seems to represent a less complete action of the drug.

Both of the units in which this effect of 10^{-4} g/ml picrotoxin was observed were lost before washing could be attempted. However, in another preparation I was able to perfuse with 10^{-5} g/ml picrotoxin and hold the cell long enough to wash with Ringer's solution. Results are shown in figure 14. This concentration of the drug produces the same kind of change in the "off" response as described above for 10^{-4} g/ml picrotoxin, but with 10^{-5} g/ml I held the cell long enough to wash with Ringer's solution and restore the "off" response to the phasic form seen before perfusion. With all concentrations of picrotoxin used, the changes became apparent within the first 5 minutes after beginning perfusion with the drug.

Picrotoxin in the concentrations used did not have a consistent effect on the light-on portion of the ocellar nerve dendrite response. In some cases the "on" response

became larger and in others smaller. Moreover, the size of the "on" response varied from light flash to light flash, with or without drugs. However, in the picrotoxin treated units the "off" overshoot in its altered form was the most reproducible part of the response, as shown in figure 15.

BICUCULLINE

The "off" response was also eliminated or greatly reduced by bicuculline in concentrations from 3×10^{-5} g/ml to 10^{-4} g/ml. This effect was seen in 4 preparations. Responses before and after bicuculline perfusion in the most stable of these preparations are shown in figure 16. Changes other than the elimination of the "off" overshoot are also evident in this unit. The noise was reduced. The sensitivity decreased by about 2 log units. The "on" transient became smaller. The potential just before the end of the 400 msec light flash, which will hereafter be referred to as the steady state potential, also decreased in amplitude after bicuculline was applied, except at the highest stimulus intensities. The changes in the light-on portion of the response developed gradually and steadily during the perfusion with bicuculline, in contrast to the apparently random fluctuations in the size of the "on" response which are normally seen. The records on the right in figure 16 were obtained after 35 minutes of perfusion with bicuculline.

By this time the responses appeared to have stabilized, and they remained essentially the same for about another 15 minutes, after which the unit was lost before washing was attempted.

Changes in the response did not begin to appear until about 15 minutes after perfusion with bicuculline was begun, and the entire effect shown in figure 16 required about 30 minutes. This may be related to the low solubility of the drug in near-neutral pH. In any case, the long perfusion time required to achieve an effect with bicuculline made replication extremely difficult. Figure 17 shows a unit in which the "off" overshoot was greatly reduced after about 15 minutes perfusion with 10^{-4} g/ml bicuculline. Perfusion with the drug was not continued in this experiment. Instead I began washing with Ringer's solution, hoping to be able to reverse the effect of bicuculline, but lost the cell soon after switching solutions. The "off" overshoot was eliminated in 2 other preparatons perfused with 3×10^{-5} g/ml bicuculline. However, both of these units showed irregularities in the light-on portion of the response before bicuculline was applied, and in one the "off" overshoot occasionally failed to appear before bicuculline was applied.

PERFUSION WITH AGONISTS AND OTHER RELATED SUBSTANCES

Since curare is an antagonist to the neurotransmitter

acetylcholine and both picrotoxin and bicuculline have been used to block GABA action, it seemed that some further understanding of the synaptic relationship between receptors and ocellar nerve dendrites might be obtained by applying the agonists and other related substances.

ACETYLCHOLINE

Two ocellar nerve dendrite preparations were perfused with 10^{-5} g/ml acetylcholine and two with 10^{-4} g/ml acetylcholine. With the lower concentration, no effect was seen. In one of the preparations perfused with 10^{-4} g/ml acetylcholine a very brief hyperpolarization was observed initially. This was immediately followed by a return toward the former resting potential, and the response to light was normal. In the other preparation, no effect of 10^{-4} g/ml acetylcholine was seen after 15 minutes of perfusion. At this point, rather than continuing to look for an effect of acetylcholine on the ocellar nerve dendrite, I thought that more information might be obtained by perfusing the preparation with a combination of acetylcholine and edrophonium, an anticholinesterase drug, since the action of the applied acetylcholine might have been prevented by hydrolysis by endogenous acetylcholinesterase.

Figure 18 shows the results of perfusion with a mixture of 10^{-4} g/ml acetylcholine and 10^{-5} g/ml edrophonium.

During a 12 minute period of perfusion with these drugs, the magnitude of the membrane potential of the ocellar nerve dendrite increased from -48 mV to -72 mV in the dark, with the exception of one brief fluctuation in the opposite direction. Along with this change, the size of the "on" transient decreased from 25 mV to 2 mV, leaving the negative peak of the "on" transient essentially the same (73 mV before the drugs and 74 mV after). The cell was lost before washing could be attempted.

Another preparation was perfused with the same mixture of 10^{-4} g/ml acetylcholine and 10^{-5} g/ml edrophonium in the presence of 10^{-3} g/ml curare. Results are shown in figure 19. There was no hyperpolarization in the dark, as was seen with acetylcholine and edrophonium alone. The resting potential remained fairly stable. The response to light began to decrease within the first three minutes and was blocked completely by 15 minutes. The unit remained stable for about another 10 minutes, after which the resting potential rapidly declined, indicating loss of the cell.

GABA

After observing the effects of picrotoxin and bicuculline on the ocellar nerve dendrite responses, I attempted to perfuse several preparations with GABA. Only one pre-

paration was held long enough to see an effect with GABA. The concentration used in this experiment was 10^{-3} g/ml. Results are shown in figure 20. During a 30 minute period of perfusion with GABA there was a gradual increase in the size of the "on" response, as well as a change in the form of the "off" response. After 2 minutes of washing the "off" response regained its oscillatory character, as shown in figure 21. Washing was continued for 6 minutes, during which there was no change in the "on" response, and at that point the cell was lost.

I was able to perfuse another preparation for about 15 minutes with 10^{-4} g/ml GABA. During most of this period there was no consistent change in the size of the "on" response, although the "off" overshoot became less sharp. However, these results are inconclusive because the resting potential began to decline immediately after GABA perfusion was begun and after 15 minutes the cell was lost.

DISCUSSION

Observations of changes in ocellar nerve dendrite membrane resistance indicate that the membrane resistance of the cell decreases during illumination (figure 6). In the great majority of chemical synapses studied thus far, transmitters have been found to decrease the postsynaptic membrane resistance by increasing the permeability to a specific ion or a number of ions (Coombs, Eccles and Fatt, 1955; Takeuchi and Takeuchi, 1960; Eccles, 1964; McLennan, 1970a). In studies of vertebrate retinas a decrease in resistance of the postsynaptic element during illumination has been taken as an indication that transmitter is released during illumination; whereas a resistance increase during illumination is interpreted as a turning off of transmitter release during illumination (Tomita, 1965; Trifonov, 1968; Toyoda, Nosaki and Tomita, 1969; Toyoda, Hashimoto and Ohtsu, 1973). This interpretation was confirmed by experiments in which vertebrate retinas were treated with high magnesium and low calcium solutions to suppress transmitter release and this treatment mimicked the effect of photostimulation on horizontal cells (Dowling and Ripps, 1973; Cervetto and Piccolino, 1974).

In contrast to this generally accepted view, the possibility of transmitters which bring about a postsynaptic

membrane potential change by turning off a membrane permeability has been raised (Kuffler, 1960; Grundfest, 1961; Krnjevic and Schwartz, 1967). This point of view has also been discussed in connection with the vertebrate retina. Kaneko (1971) has suggested that the response of the "on" center bipolar cells of the dogfish retina may be mediated by a receptor transmitter which closes channels in the subsynaptic membrane of the bipolar cell. Toyoda (1973) suggests a similar mechanism for the response of the "on" center bipolar cells of the carp retina, but points out that no transmitter is known to act by this means. Gershenfeld and Paupardin-Tritsch (1974) have recently reported that iontophoretic application of 5-hydroxy-tryptamine to neurons of _Helix_ and _Aplysia_ cause a depolarization accompanied by a decrease in potassium conductance and a hyperpolarization accompanied by a decrease in sodium and chloride conductance. The authors point out that the ionic channels may exist in an open conformation in the membrane until acted upon by 5-hydroxytryptamine, or alternatively, that they remain open as a result of continuous action of a transmitter and that the applied 5-hydroxytryptamine may in some way interfere with this action.

While a transmitter which acts by decreasing permeability to specific ions and thus increases membrane resis-

tance seems to be a logical possibility, an actual transmitter that has this effect has not yet been described. Thus it seems most reasonable at this time to assume that the transmitters in the dragonfly ocellus also act by decreasing postsynaptic membrane resistance, and, therefore, that the receptor transmitter is released upon illumination. This assumption is especially reasonable since curare blocks the response at light-on while acetylcholine hyperpolarizes the ocellar nerve dendrite.

Thus, the hyperpolarizing ocellar nerve dendrite response, although it is similar to the response of the "off" center amacrine cells in the carp retina, appears to result from the release of an inhibitory transmitter upon illumination, as suggested by Chappell and Dowling (1972), rather than from the turning off of an excitatory transmitter which is released in the dark. The latter mechanism was proposed by Toyoda, Hashimoto and Ohtsu (1973) for the "off" center amacrine response, on the basis of resistance measurements which showed that the hyperpolarizing response of those cells was accompanied by a resistance increase. The mechanism found in the ocellus may be more relevant to the lamina (first synaptic plexus) of the insect compound eye, where the receptors are depolarized by light (Autrum and Kolb, 1968, 1972; Horridge, 1969; Eguchi, 1971) while the

monopolar neuron (second order) is hyperpolarized (Zettler and Jarvilheto, 1971; Laughlin, 1973).

The experiments shown in figures 7 through 21 are concerned with the response of ocellar nerve dendrites to light. This response appears to be blocked by curare and mimicked by acetylcholine. The lowest concentration of curare which blocked the ocellar nerve dendrite response in this study was 10^{-4} g/ml. This is comparable to concentrations used in other perfused preparations such as the neuromuscular junction where Dale, Feldberg and Vogt (1936) used 2×10^{-5} g/ml curare to block muscular contraction, and the sixth abdominal ganglion of the cockroach, in which Kerkut, Pitman and Walker (1969) found that 10^{-4} g/ml curare is the threshold concentration required to antagonize nicotine.

Dale (1914) first proposed the existence of two pharmacologically distinct classes of cholinergic actions, one class which could be mimicked by application of nicotine and antagonized by curare, and another which could be mimicked by muscarine and antagonized by atropine. In vertebrates, the neuromuscular junction is nicotinic, while the synapses of the parasympathetic innervation of effector organs such as exocrine glands, smooth muscle, and cardiac muscle are muscarinic. However, there is experimental evidence that at

least some vertebrate neurons have both types of acetylcholine receptors, as found in sympathetic ganglion cells (Eccles and Libet, 1961) and spinal Renshaw cells (Curtis and Ryall, 1966). Invertebrate neurons also appear to have both types of acetylcholine receptors. Tauc and Gerschenfeld (1961) report that the effects of applied acetylcholine on the D cells and H cells in Aplysia are diminished or abolished by 10^{-4} g/ml curare or atropine. Although my preparation showed a greater sensitivity to curare than to atropine in the concentrations used, the atropine perfusion was successfully carried out on only one preparation, so the observed results must be considered somewhat tentative.

The blocking of the ocellar nerve dendrite response by curare raises the question of the sensitivity of these cells to applied acetylcholine. Perfusion with acetylcholine caused a brief hyperpolarization of these cells, but although the potential change was in the same direction as the normal ocellar nerve dendrite response, it was of such brief duration that further analysis was impossible. At somatic neuromuscular junctions of vertebrates, the action of acetylcholine is terminated by hydrolysis of the transmitter by the enzyme acetylcholinesterase (Brown, Dale and Feldberg, 1936; Fatt and Katz, 1952). This enzyme can be blocked by a group of anticholinesterase drugs, with the result that

acetylcholine accumulates and its synaptic action is potentiated. These drugs include eserine (physostigmine), neostigmine (prostigmine), and edrophonium (tensilon) (Katz, 1966). At high concentrations most anticholinesterase agents can also block acetylcholine by combining with acetylcholine receptors (Katz, 1966). Goldsmith (1963) discusses the problem of specificity of action and reports that edrophonium is a virtually specific anticholinesterase at concentrations below 10^{-4} g/ml.

When I added 10^{-5} g/ml edrophonium to the perfusion fluid to prevent hydrolysis of the applied acetylcholine, a sustained effect was observed which closely mimicked the effect of the receptor transmitter. The ocellar nerve dendrite membrane potential became progressively more negative, reaching a maximum of -72 mV within 12 minutes, essentially matching the light-induced hyperpolarization of -73 mV. The one fluctuation of the potential in the opposite direction during this period is not readily explained, but the hyperpolarization is unquestionably the dominant effect. When light flashes were presented during this period of hyperpolarization, the ocellar nerve dendrite response was smaller than before the drugs were applied, and in no case did the negative peak of the "on" transient exceed -74 mV. Thus it appears that both the applied

acetylcholine and the receptor transmitter drive the ocellar nerve dendrite membrane potential to a level around -72 to -74 mV.

In the experiment discussed above, a mixture of two drugs, acetylcholine and edrophonium, was used. This raises the question of the validity of attributing the observed changes to acetylcholine, since there was no control experiment in which edrophonium alone was applied. However, although the results of such an experiment would be of interest, it would not be an unambiguous control. If edrophonium alone had had no effect, the obvious conclusion would have been that the changes seen with acetylcholine and edrophonium were due to acetylcholine. However, if edrophonium alone caused changes of the kind seen with the mixture of acetylcholine and edrophonium, or if edrophonium alone increased or prolonged the response to light, these results could be attributed to a potentiation of the effect of acetylcholine released by the receptors in the dark in the former case or of acetylcholine released in response to light in the latter. Thus, all of these possible results would tend to support a cholinergic mechanism.

The pharmacological similarity between acetylcholine and the receptor transmitter is demonstrated by the experiment shown in figure 19. Here curare, in a concentration

which produced a fast, reversible block of the light response in earlier experiments, blocks both the response to light and the hyperpolarizing response to applied acetylcholine and edrophonium.

The concentration of acetylcholine used in the experiments discussed above, 10^{-4} g/ml, is approximately equivalent to 10^{-3} M, and is comparable to concentrations needed to affect transmission across intact insect ganglia. Yamasaki and Narahashi (1958) reported that 10^{-2} M acetylcholine was needed to discharge impulses in the last abdominal ganglion of the cockroach, while the threshold concentration was reduced to 10^{-3} M when the ganglion was either desheathed or pretreated with eserine. Similarly, Kerkut, Pitman and Walker (1969) report thresholds of 5×10^{-5} g/ml to 5×10^{-3} g/ml to bath-applied acetylcholine in cockroach ganglia. These authors used 10^{-5} g/ml eserine to potentiate the effect of acetylcholine. They also used edrophonium for which no concentration was given, although they note that their preparation was less sensitive to edrophonium than to eserine.

In contrast, when acetylcholine is applied iontophoretically to insect (Kerkut, Pitman and Walker, 1969) and molluscan (Gerschenfeld and Stefani, 1967) preparations, rather than perfused, thresholds as low as 10^{-13} M and

10^{-14} M respectively have been reported. Thus a barrier apparently exists to acetylcholine in intact insect preparations, and the relatively high concentrations used in the experiments in this study are comparable with those found necessary by other investigators working with similar preparations. A similar situation appears to exist in vertebrate preparations. The threshold for acetylcholine applied by droplet to single nerve-muscle fibers in frog sartorius was found to be about 10^{-6} M (Kuffler, 1943), but with iontophoretic application to a frog neuromuscular junction preparation a threshold of less than 10^{-15} M was reported (del Castillo and Katz, 1955).

The experiments discussed thus far provide some useful information about the ocellus. First, taking a most conservative point of view, curare may provide a simple means of isolating the receptor response in the ocellus and may make it possible to record receptor responses unmodified by feedback from ocellar nerve dendrites. In vertebrate retinas aspartate and glutamate have been used to isolate receptor responses (Cervetto and MacNichol, 1972; Murakami, Ohtsu and Ohtsuka, 1972), although the mechanism is different, since aspartate and glutamate are thought to mimic the action of a receptor transmitter which is turned off by illumination, rather than to block a receptor transmitter which is released by illumination.

Curare is widely accepted as a specific cholinergic antagonist, and its blocking action in the ocellus raises the possibility of cholinergic transmission. However, curare has been found to antagonize the action of 5-hydroxytryptamine on certain molluscan neurons (Gerschenfeld and Paupardin-Tritsch, 1974).

An additional piece of evidence in favor of cholinergic transmission in the ocellus was obtained in some preliminary experiments in which assays of ocellar tissue indicated a significant amount of acetylcholine and of choline acetyltransferase activity (Kuhar and Chappell, 1975). Although the identity of the receptor transmitter in the ocellus is still in question, the results here raise acetylcholine to the status of a most promising candidate for that role.

The "off" overshoot portion of the ocellar nerve dendrite response can be abolished by picrotoxin and bicuculline, and in one particularly stable preparation a long period of perfusion with bicuculline affected all parts of the response. Several considerations arose in interpreting these results.

I attempted to rule out the possibility that the drugs were acting at some site in the brain rather than at the receptor-ocellar nerve dendrite synaptic region, by tying,

cutting, or crushing the ocellar nerve just before it entered the brain, but the nerve is very short (about 1 mm) and after tying or crushing it there was literally nothing left. Cutting the nerve was also unsuccessful. Normally when the ocellar nerve is attached to the protocerebrum it is held under some tension. When the nerve is cut at the proximal end it retracts toward the receptor structure, rolls under it, and floats in the surrounding Ringer's solution, making it impossible to drive the microelectrode into it. However, although it was not possible to limit the site of action of the drugs by such a direct approach, it may be assumed that they are acting at the synaptic region of the ocellus. Chappell and Dowling (1972) found that tetrodotoxin did not alter the slow potential activity of the ocellar nerve dendrites. Since any centrifugal influence from the brain on the ocellar nerve dendrite response may be expected to depend on impulse conduction, the effects of bicuculline and picrotoxin may be attributed to actions at the receptor-ocellar nerve dendrite synaptic region.

Both picrotoxin and bicuculline have been widely used to antagonize the effects of GABA on neurons, and to block synaptic transmission believed to be mediated by GABA. This amino acid was shown by Bazemore, Elliott and Florey (1957) to have an inhibitory action on crayfish stretch receptor

neurons. Since then, convincing evidence has been presented that GABA mimics the effect of inhibitory stimulation at that site (Kuffler and Edwards, 1958) and at the crayfish neuromuscular junction (Takeuchi and Takeuchi, 1965). The presence of GABA (Kravitz, Kuffler and Potter, 1963) and of the enzymes and substrates of GABA metabolism (Kravitz, Molinoff and Hall, 1965) have been demonstrated in the inhibitory axons at the crayfish neuromuscular junction, as well as the release of GABA by stimulation of the inhibitory presynaptic axons (Otsuka, Iverson, Hall and Kravitz, 1966). GABA appears to have a similar inhibitory effect on insect muscle (Usherwood and Grundfest, 1965) and insect ganglia (Suga and Katsuki, 1961; Kerkut, Pitman and Walker, 1969; Pitman and Kerkut, 1970). As discussed above in connection with acetylcholine, there also appears to be a barrier to perfused GABA in intact insect ganglia. Suga and Katsuki (1961) report that 10^{-2} g/ml GABA (approximately equivalent to 10^{-1} M applied by perfusion was required to completely block transmission at the auditory synapse of the grasshopper, although lower concentrations brought about a decrease in activity. In contrast, GABA applied iontophoretically to insect central neurons was effective in concentrations of 1.05×10^{-13} M (Kerkut, Pitman and Walker, 1969).

The presence of GABA in mammalian brain and its inhibitory action in invertebrates (Bazemore, Elliott and Florey, 1957) stimulated investigation of its effect on various vertebrate preparations. Curtis, Phillis and Watkins (1959) found that GABA had a depressant action on spinal neurons but pointed out that it differed from the transmitter causing postsynaptic inhibition in that the latter was antagonized by strychnine while the action of GABA was not. However, the strychnine-resistant postsynaptic inhibition of the cerebral cortex (Krnjevic and Schwartz, 1967) and inhibition of the neurons of Dieter's nucleus by the Purkinje cells of the cerebellar cortex (Obata, Ito, Ochi and Sato, 1967) are both closely mimicked by GABA.

There is also evidence that presynaptic inhibition in the spinal cord is mediated by GABA. Frank and Fuortes (1957) applied the term presynaptic inhibition to the action of muscle afferent volleys in reducing the size of the monosynaptic excitatory postsynaptic potential (EPSP) of motoneurons. This action is attributed to a diminished excitatory synaptic action of the presynaptic impulses (Eccles, Kostyuk and Schmidt, 1962). Eccles, Schmidt and Willis (1963) found that GABA mimicked this effect and suggested that it might act by depolarizing the central terminals of primary afferents, thus presumably reducing

their synaptic action. Recent work in the frog spinal cord indicates that GABA depolarizes primary afferents in that preparation (Barker and Nicoll, 1972; Davidoff, 1972). Barker and Nicoll (1972) also report that the mechanism of GABA action in this case involves sodium ion rather than chloride, as in the invertebrate actions and vertebrate postsynaptic inhibitory actions of GABA.

Picrotoxin blocks GABA at many invertebrate sites, including the neuromuscular junctions of most crustaceans and insects, insect central neurons, and crustacean stretch receptors and cardiac ganglia (Robbins, 1959; Usherwood and Grundfest, 1965: Pitman and Kerkut, 1970; Curtis and Watkins, 1966). In the vertebrate central nervous system, picrotoxin has been reported as an effective GABA antagonist in the cuneate nucleus of the cat (Galindo, 1969), in Dieter's nucleus (Obata, Takeda and Shinozaki, 1970), in the spinal cord (Engberg and Thaller, 1970), in the olfactory bulb (Nicoll, 1971), and in the cerebellum (Woodward, Hoffer, Siggins and Oliver, 1971). However, inconsistencies in the effectiveness of picrotoxin as a GABA antagonist in vertebrate central nervous system are discussed by Curtis, Duggan, Felix and Johnston (1971). The specificity of picrotoxin as a GABA antagonist is further complicated by the report that it antagonizes glycine inhibition (Davidoff and Aprison, 1969).

The alkaloid bicuculline, known to have convulsant properties (Welch and Henderson, 1934), has recently been employed as a GABA antagonist at several sites, including the crayfish stretch receptor (McLennan, 1970b), the cat central nervous system (Curtis, Duggan, Felix and Johnston, 1970a, 1970b, 1971; Curtis, Duggan, Felix and McLennan, 1971; Curtis and Felix, 1971; Bisti, Iosif, Marchesi and Strata, 1971), and the frog spinal cord (Barker and Nicoll, 1972; Davidoff, 1972). Bicuculline was shown to competitively inhibit binding of GABA by synaptosomal fractions of rat cerebellar cortex (Peck, Schaeffer and Clark, 1973; Schaeffer, Clark and Peck, 1974). Although there is now much evidence that bicuculline is a GABA antagonist, it must be noted that some investigators have found it ineffective against GABA (Godfraind, Krnjevic and Pumain, 1970; Straughan, Neal, Simmonds, Collins and Held, 1971), and that recent evidence indicates that it can also act as an anticholinesterase (Svenneby and Roberts, 1973; Miller and McLennan, 1974).

The concentrations of picrotoxin and bicuculline used here to alter the ocellar nerve dendrite response are comparable to those used in other invertebrate preparations. Usherwood and Grundfest (1965) and Pitman and Kerkut (1970) used 10^{-4} g/ml picrotoxin to block both IPSPs and the response

to applied GABA in insect muscle and insect central neurons respectively. McLennan (1970b) reported that bicuculline in concentrations ranging from 3 x 10^{-5} to 3 x 10^{-4} g/ml reduced or prevented the inhibitory action of GABA on crayfish stretch receptor, and noted that bicuculline was about 10 times more potent (by weight) than picrotoxin in that preparation.

The most straightforward explanation of the effects of picrotoxin and bicuculline on the ocellar nerve dendrite response would be that the drugs are blocking feedback from the ocellar nerve dendrites to the receptors. If small amounts of both the receptor transmitter and the feedback transmitter are normally released in the dark, the overshoot and oscillations at light-off could result from the time lag between the cessation of the light stimulus and the return of the transmitters to their dark-adapted equilibrium, and blocking the feedback transmitter could eliminate these oscillations. Before the present study was begun, it seemed most likely that the synapse made by the ocellar nerve dendrites onto receptor terminals would have an inhibitory or negative feedback function analogous to the lateral inhibition in *Limulus*, as suggested by Dowling and Chappell (1972). Toyoda, Hashimoto and Ohtsu (1972) also discuss the possibility of negative feedback in the carp retina and point out that the initial transients of many amacrine cell

responses could be accounted for by inhibitory or negative feedback from amacrine cells to bipolar cells.

If this type of negative feedback occurred in the ocellus, transmission from the receptor terminal to the ocellar nerve dendrite would turn on a process in the latter which would turn down the transmission from the receptor to the second order cell. This model predicts that blocking the feedback would result in an increase in the size of the ocellar nerve dendrite response, since transmission from the receptor terminal would no longer be turned down. However, in the experiment shown in figure 16 in which an ocellar nerve dendrite preparation was perfused with bicuculline for almost an hour, several stable effects appeared which are inconsistent with such a negative feedback model. Both the "on" transient and the steady state potential decrease in size after perfusion with bicuculline, with the exception of the steady state potential at the two highest intensities. Another unexpected observation was that after perfusion with bicuculline there was a decrease in sensitivity of about 2 log units.

These observations suggested another model for the feedback from the ocellar nerve dendrite to the receptor terminal, one in which the ocellar nerve dendrite exerts a facilitory influence on the receptor terminal; that is, it

results in an increased rate of release of receptor transmitter. The facilitory feedback functions in the dark, and is reduced or turned off during illumination, when the ocellar nerve dendrite is hyperpolarized. Figure 22 shows how such a model could account for the typical features of the ocellar nerve dendrite response.

According to this model, in the dark adapted condition (A) a certain amount of receptor transmitter, represented by the downward arrows, is released by the receptor terminal and a certain amount of feedback transmitter, represented by the upward arrows, is released by the ocellar nerve dendrite. The numbers of arrows are arbitrarily chosen to represent amounts of transmitter relative to the dark equilibrium condition. The receptor transmitter hyperpolarizes the ocellar nerve dendrite and reduces its output of feedback transmitter, while the latter increases the receptor terminal's output of its transmitter. The dark adapted condition represents an equilibrium between these two cells such that transmitter is released by each cell at a fairly steady rate and a stable "resting" potential is maintained by each cell. The noise which is often recorded in the ocellar nerve dendrite in the dark may represent small variations in the amount of receptor transmitter released as this equilibrium is maintained. At light-on (B), the receptor is depolarized and releases more transmitter, which

hyperpolarizes the ocellar nerve dendrite. Shortly after light-on (C), the ocellar nerve dendrite, which is now hyperpolarized, is releasing little or no feedback transmitter, and thus the receptor terminal is less facilitated than it was just before light-on. (The nature of this facilitation will be discussed below.) Therefore the receptor terminal's output of transmitter is reduced and the ocellar nerve dendrite potential returns toward the dark level. Eventually an equilibrium or steady state (D) is reached in which the receptor terminal is releasing <u>less</u> transmitter than immediately after light-on (due partly to receptor properties and partly to decreased facilitation by the ocellar nerve dendrite) but <u>more</u> than in the dark, resulting in a small hyperpolarization of the ocellar nerve dendrite. At light-off (E), the receptor potential returns toward the resting level and the output of receptor transmitter is greatly reduced, and consequently the ocellar nerve dendrite potential also returns toward its resting level. However, just after light-off there is another factor operating in addition to the cessation of light-induced release of receptor transmitter. Since the ocellar nerve dendrite was somewhat hyperpolarized just before light-off, less feedback transmitter was released than in the dark adapted condition, and therefore at light-off there is

less feedback transmitter available to facilitate receptor transmitter release than in the dark adapted condition. At some point after light-off the release of receptor transmitter falls below the dark adapted level and the ocellar nerve dendrite potential briefly overshoots the dark adapted level with a resultant increase in the amount of feedback transmitter released. This is followed by a period of variable oscillations (F) which may include overshoots and undershoots of the resulting potential, as the original equilibrium between the two cells is reestablished (G).

The effect of bicuculline on this system is presented in figure 23. In the dark adapted condition (A) bicuculline blocks the action of feedback transmitter on the receptor terminal. This in turn prevents the dark release of receptor transmitter, which should cause a shift in the resting potential of the ocellar nerve dendrite in the positive direction. The significance of this shift will be discussed more fully below. The decrease in noise recorded in the ocellar nerve dendrite in the dark after perfusion with bicuculline (figure 16) may be due to the cessation of dark release of receptor transmitter when the feedback transmitter is blocked. At light-on (B), the receptor is depolarized and releases a certain amount of transmitter, but without the facilitory effect of the feedback the light-

induced ocellar nerve dendrite hyperpolarization is smaller in magnitude than it is in the normal case. In terms of the schematic model, even though the light-induced receptor depolarization may be the same, the increment in receptor transmitter release in the presence of bicuculline is represented by 4 arrows, whereas in the normal case 5 additional arrows of receptor transmitter are released at light-on. This non-linearity will be discussed further below. As the light remains on, the transmitter output of the receptor decreases somewhat due to processes in the receptor itself, and the ocellar nerve dendrite potential returns toward the dark level, but the return is slower than in the normal case. The steady state potential (C) may be smaller in size than in the normal case, particularly at lower light intensities where the effect of the facilitating feedback on transmitter release by the receptor is particularly important compared to the effect of light. At light-off (D), the release of receptor transmitter declines and ceases as the receptor terminal returns to the dark potential, and the ocellar nerve dendrite correspondingly returns to its dark potential without overshoots or oscillations.

Figure 24 shows the potentials recorded in the ocellar nerve dendrite in the normal and the bicuculline treated cases. The facilitory model predicts that bicuculline should cause the resting potential to shift to the level

reached by the "off" overshoot in the normal case. The direction of the change in resting potential upon perfusion with bicuculline might be expected to distinguish between the facilitory and inhibitory models, since blocking a facilitory feedback would decrease dark release of receptor transmitter and thus shift the ocellar nerve dendrite dark potential in the positive direction, whereas blocking an inhibitory feedback would increase dark release of receptor transmitter and cause a negative shift in the ocellar nerve dendrite dark potential (release of transmitters in the dark being necessary to account for oscillations in the ocellar nerve dendrite potential after light-off). However, in two bicuculline treated preparations in which resting potentials were monitored, results were not conclusive. In one (figure 16) the resting potential began shifting in the negative direction before bicuculline perfusion was started, and this continued during perfusion with the drug. In the other preparation (figure 17) the resting potential shifted 8 mV positive after bicuculline perfusion was begun. In this preparation, the maximum "off" overshoot before bicuculline was 8 mV, and the overshoot was abolished by bicuculline at all but the highest stimulus intensity. The latter experiment lends support to the facilitory model, while the former appears inconclusive on this particular point, since the

shift in the resting potential began before the drug was applied. Such increases in the size of the resting potential have been observed in other cells not treated with drugs, and are thought to be related to recovery of the cell membrane after penetration by the microelectrode. It must be added that positive-going resting potential drifts are also commonly observed, and are often followed by loss of the cell. According to the same reasoning, curare should block the effect of dark-released receptor transmitter and thus cause a positive shift in the ocellar nerve dendrite resting potential. Resting potential was monitored in two experiments in which curare blocked the ocellar nerve dendrite response. In one, a positive shift of 4 mV occurred within the first 3 minutes after curare perfusion was begun. In the other, no shift in the resting potential was observed. However, the fact that the shift in the resting potential may not be observed in every case is inconclusive, since the recording site may be some distance away from the site of transmitter action and the passive decay of the synaptic potential may be considerable.

To return briefly to the alternative model, an inhibitory negative feedback system, with both transmitters being released in the dark, could also generate all the features of the normal response. However, as discussed

above, it is difficult to explain the decrease in ocellar nerve dendrite response size and sensitivity upon blocking feedback if the effect of feedback on the receptor terminal is inhibitory. The inhibitory feedback model would also imply that the ocellar nerve dendrite releases feedback transmitter when it is hyperpolarized.

The one effect of bicuculline which is difficult to reconcile with the facilitory model is the slow return to the resting potential at light-off at the two highest intensities (figure 16). This effect appears to be more consistent with the inhibitory feedback model, in which case it could be argued that with the inhibitory feedback blocked by bicuculline there is so much receptor transmitter released during illumination at the higher intensities that some of it is still available after light-off. The increase in size of the steady state potential at these same intensities is qualitatively consistent with this interpretation. However, recent reports have indicated that bicuculline, in addition to blocking GABA, can also act as an anticholinesterase (Svenneby and Roberts, 1973; Miller and McLennan, 1974). An alternative explanation could be that this action becomes significant at higher stimulus intensities, when larger amounts of receptor transmitter are presumably released, accounting for the slow return to baseline at light-off. This hypothesis is consistent with other evidence

pointing toward cholinergic transmission. In view of these findings it would be interesting to compare the effect of bicuculline on the ocellar nerve dendrite response with that of other anticholinesterases. Whatever the actual explanation for the slow return after light-off with higher intensities in the presence of bicuculline, it is interesting to note that when the feedback is presumably blocked the response in the second order cell mirrors the receptor response more faithfully. When feedback is intact, the ocellar nerve dendrite response at these intensities approximates the time course of the stimulus in spite of the slow return of the receptor potential which is typically recorded at the higher stimulus intensities, as shown in figure 5.

The model presented above implies that the facilitory feedback is responsible for both the release of receptor transmitter in the dark and the enhancement of light-evoked output of receptor transmitter without specifying the mechanism of this facilitation. Any attempt to suggest a possible mechanism at this time is necessarily highly speculative, and requires certain assumptions about how synaptic transmission is controlled by slow potential changes. There have been several studies of the relation between presynaptic polarization and synaptic transmission, but all have employed preparations in which transmission is normally evoked by

impulses, even though impulses may have been artificially blocked in the course of the experiment. In the dragonfly ocellus transmission between receptors and ocellar nerve dendrites appears to be evoked by slow potentials, since it is not altered by tetrodotoxin, which eliminates impulse activity in the receptors (Chappell and Dowling, 1972) and in the ocellar nerve (Gallin and Chappell). Although this type of transmission has not been extensively studied, it appears to be quite significant in vertebrate retinas (Werblin and Dowling, 1969; Kaneko, 1970; Murakami and Shigematsu, 1970), and probably in the brain.

The simplest explanation consistent with the data presented above is that in the dragonfly ocellus release of transmitters by both receptor terminals and ocellar nerve dendrites is directly related to depolarization of the presynaptic membrane, and that the relation between amount of depolarization and transmitter release is non-linear. In the absence of a clear understanding of how transmitter release is controlled by slow potential changes alone, a careful consideration of the available literature lends some support to the assumption of a direct relation between depolarization and transmitter release.

Firstly, there is much evidence that where transmission is evoked by impulses, the size of the postsynaptic potential

depends upon the size of the presynaptic spike, both at the squid giant synapse (Hagiwara and Tasaki, 1958; Takeuchi and Takeuchi, 1962) and at the synapse of the primary afferent fibers on motoneurons in the cat spinal cord (Eccles, Kostyuk and Schmidt, 1962). In these cases, a suitably timed preceding depolarization of the presynaptic terminal decreases the size of the presynaptic action potential and of the postsynaptic potential which that action potential evokes, while hyperpolarization causes an increase in the size of the presynaptic action potential and in the postsynaptic potential. The interaction of the preceding depolarization or hyperpolarization with the presynaptic impulse is believed to result in presynaptic inhibition (Eccles, Kostyuk and Schmidt, 1962) and presynaptic facilitation (Mendell and Wall, 1964; Kandel and Tauc, 1965) respectively. In these cases, the presynaptic polarizations exert their influence on transmitter release indirectly, by means of their effect on the presynaptic action potential.

On the other hand, when presynaptic impulses are prevented experimentally, either by the use of slowly rising currents or tetrodotoxin, the effect of the applied polarization *per se* is seen. In this situation, transmission, as measured by size of the postsynaptic potentials at the squid giant synapse (Bloedel, Gage, Llinas and Quastel,

1966; Katz and Miledi, 1966) and by frequency of miniature post-synaptic potentials at the vertebrate neuromuscular junction (del Castillo and Katz, 1954; Liley, 1956) is augmented by presynaptic depolarization and decreased by presynaptic hyperpolarization. In the study by Liley, this was found to hold not only for brief pulses but for depolarizations lasting up to 5 minutes, during which the frequency of miniature postsynaptic potentials remained stable. This same study also produced evidence that the frequency of miniature potentials increased logarithmically with the amount of depolarization.

The application of these complex findings to the synaptic region of the dragonfly ocellus requires caution. However, the studies in which presynaptic impulses were blocked and transmission was evoked directly by applied pulses seem most relevant to the ocellar synapses, where transmission is evoked by slow potentials. Liley's findings are of particular interest. The model presented above requires that the relation between membrane potential and transmitter release in the ocellus be stable over long periods. According to the model, it is the absolute level of membrane potential that determines the amount of transmitter released. Furthermore, Liley's observation that the frequency of miniature potentials increased logarithmically

with the amount of depolarization suggests a possible mechanism for the apparent facilitating effect of the feedback transmitter. According to the facilitory model, the feedback transmitter which is released in the dark could bring about a depolarization of the receptor terminals which results in the release of some receptor transmitter in the dark. In addition, the fact that this feedback is operating in the dark would cause the amount of receptor transmitter released in response to a given light stimulus to be greater than it would be without feedback. (The decrease in size of the "on" response after treatment with bicuculline is shown in figure 16. The same phenomenon is represented schematically by an increment of 5 "arrows" of receptor transmitter at light-on in figure 22, compared with an increment of 4 "arrows" in figure 23 when feedback is blocked.) When the light-induced slow depolarizing potential of a receptor cell reaches the receptor axon terminal it presumably causes the release of a certain amount of transmitter. If the relationship between depolarization and transmitter release here is not linear but closer to the logarithmic relation reported by Liley at the rat diaphragm neuromuscular junction, a given light-induced depolarization would be more effective if it were superimposed on a pre-existing depolarization of the receptor terminals caused by the feedback transmitter.

Such an effect could conceivably involve a more efficient mobilization of transmitter when the terminal is already somewhat depolarized.

Although the results obtained with picrotoxin and bicuculline are insufficient to draw firm conclusions as to the identity of the feedback transmitter, particularly in view of questions which have been raised about their specificity, the widespread use of these two drugs as GABA antagonists raises the possibility that GABA may be the feedback transmitter.

The one preparation in which a GABA perfusion was completed showed an increase in the size of both the "on" transient and the steady state response, an effect which is consistent with a facilitory function. No change in the resting potential was seen in this experiment, but, as discussed above, the effects of synaptic feedback, of bicuculline, and of GABA on the resting potential, although they may be significant at the subsynaptic portions of the ocellar nerve dendrite membrane, may not be large enough to be detected at the recording sites in these experiments. Although the change in the ocellar nerve dendrite response with GABA was in the direction predicted by the facilitory model, it should be pointed out that the response before GABA was unusually small (10 mV maximum), and the increase

in size with GABA only brought the response to 22 mV which is more comparable to a "typical" ocellar nerve dendrite response. However, it is unlikely that the changes observed after perfusion with GABA are due simply to recovery of the unit from damage by the microelectrode, since the resting potential was -38 mV immediately after entering the cell and remained at that level through more than 30 minutes of GABA perfusion and 6 minutes of washing with Ringer's solution, after which the cell was lost. The relatively high concentration of GABA (10^{-3} g/ml) used in this experiment is comparable with concentrations found necessary in other perfused insect preparations, as noted above.

Perfusion of another preparation with 10^{-4} g/ml GABA was attempted, but both the resting potential and the response to light declined steadily and eventually disappeared. This occurs commonly in intracellular experiments and probably results from damage to the cell membrane during penetration with the microelectrode.

The effect of picrotoxin (figures 11 through 15), of GABA (figures 20 and 21) and the effect of bicuculline on the preparation shown in figure 17 indicate that the "off" overshoot is the most pharmacologically sensitive portion of the ocellar nerve dendrite response. This is reasonable in light of the model presented above, since the

"off" response results from a dynamic interplay between the receptor and feedback transmitters, and a partial block of the feedback transmitter may have a great effect on this portion of the ocellar nerve dendrite response, while the same partial block may not significantly alter the amount of receptor transmitter released at light-on.

Although picrotoxin and bicuculline have similar effects on the "off" response, their effects on the light-on portion of the ocellar nerve dendrite response are more difficult to evaluate. In the picrotoxin experiments, the size of both the transient and steady state portions of the "on" response increased in some units and decreased in others after perfusion with the drug, and there was much variability in response size from one flash to another. However, in the one bicuculline-treated unit which was held for over an hour (figure 16), there was a clear reduction in size of the transient and steady state "on" response. Although this reduction in response size, along with a decrease in sensitivity, was observed in only one unusually stable unit, it constitutes the best evidence to date of the effect of bicuculline on the ocellar nerve dendrite "on" response, and has been incorporated into the model.

In summary, the results of the present study indicate that the photoreceptors of the dragonfly ocellus release a

transmitter substance upon illumination which has an inhibitory effect on the ocellar nerve dendrites, inhibiting both the impulse activity which occurs in the dark and the release of feedback transmitter by the ocellar nerve dendrites. There is evidence that the receptor transmitter may be acetylcholine, but further pharmacological investigation is necessary to test this.

The results also indicate that the effect of the ocellar nerve dendrite feedback transmitter on receptor terminals is facilitory and suggest that this transmitter could be GABA. Illumination of the ocellus is presumed to block release of this feedback transmitter. An inhibitory feedback would seem inappropriate here, where input from the receptors to the ocellar nerve dendrites is inhibitory.

The facilitory feedback loop proposed, with both transmitters being released in the dark and modified by illumination, could give rise to the "off" overshoot and the burst of impulses associated with it. The effects of bicuculline, picrotoxin, and GABA on the ocellar nerve cells can be further investigated through their effects on the pattern of impulse activity in the ocellar nerve. Such extracellular experiments are technically much less difficult, and together with intracellular investigations such as the present study, may contribute to an understanding of how the ocellus processes information for transmission to the brain.

Figure 1

Dissected dragonfly head mounted in perfusion apparatus and ready for intracellular recording. (a) dissected dragonfly head; (b) cork; (c) plexiglass chamber; (d) cotton wick; (e) reference electrode; (f) microelectrode; (g) 30 guage needles; (h) plastic tubing; (i) valves; (j) 10 ml syringes.

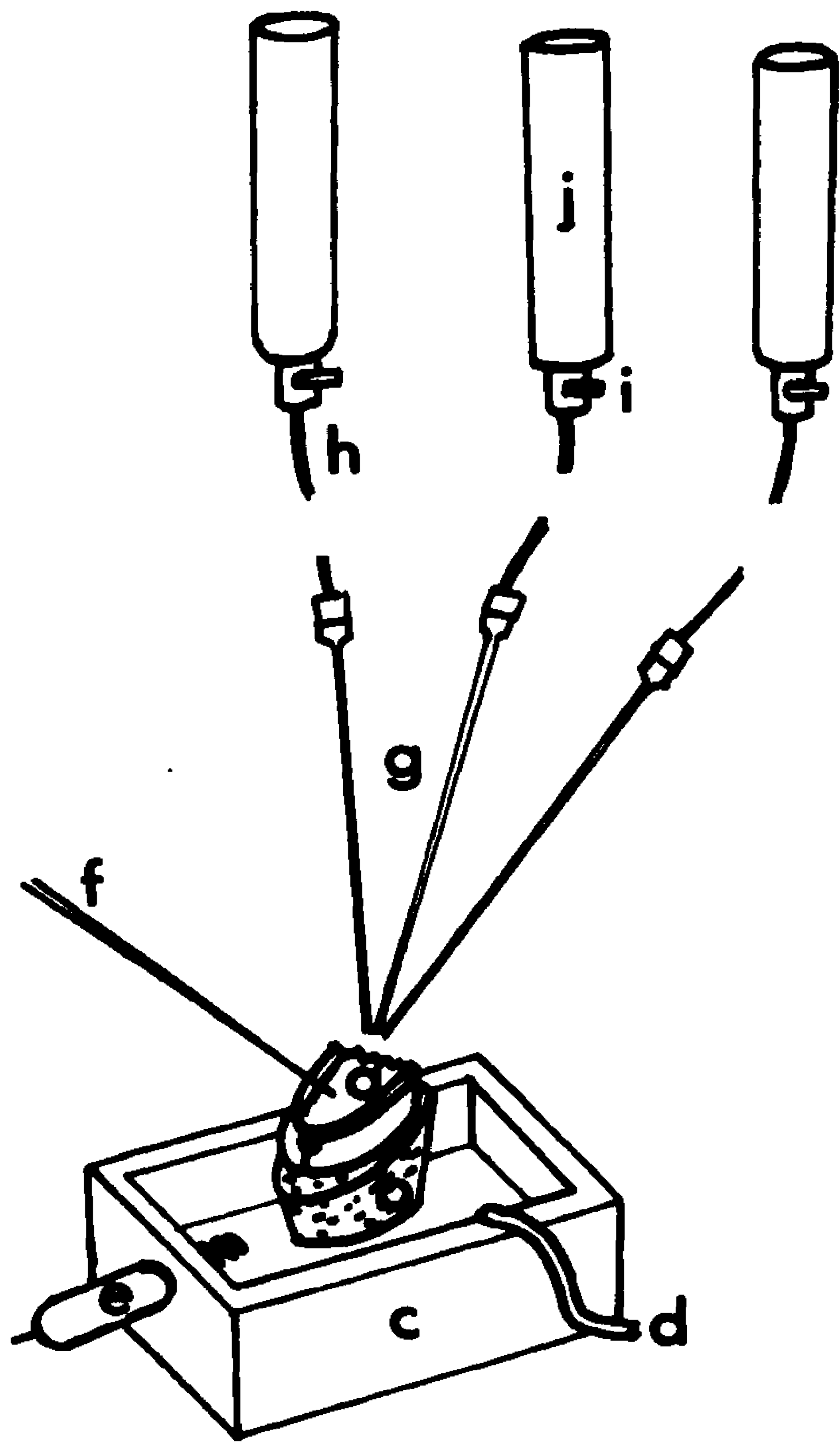

j
h
i
g
f
e
c
d

Figure 2

Oscilloscope traces obtained by passing trains of 1 nAmp depolarizing current through a microelectrode in Ringer's solution. The downward transients mark the beginning of the pulses.

Upper trace: the Mentor N-950 bridge potentiometer was adjusted for a null reading.

Middle trace: the bridge potentiometer has been set at a higher value than that which gives a null reading, causing a downward displacement of the pulse-on portion of the trace. A similar displacement would be seen if the potentiometer setting had not been changed but the electrode resistance had decreased.

Lower trace: the potentiometer has been set at a lower value than that which gives a null reading, causing an upward displacement of the pulse-on portion of the trace. A similar displacement would be seen if the electrode resistance had increased.

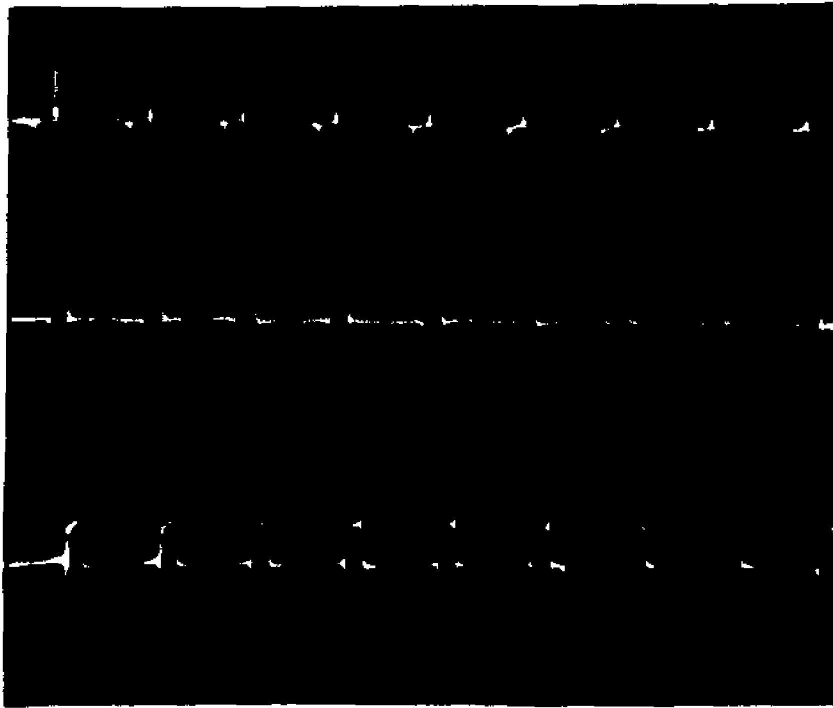

10 mV
.1 sec

Figure 3

Response of an ocellar nerve dendrite in which impulse activity was recorded. Impulses are inhibited in the light, at which time the ocellar nerve dendrite is hyperpolarized. During the overshoot of the resting potential after light-off, there is a burst of impulses at a higher frequency than in the dark adapted condition.

Log I = -2.

The horizontal line at the botton indicates duration of the stimulus.

Recorded from *Aeschna tuberculifera*.

5 mV

.1 sec

Figure 4

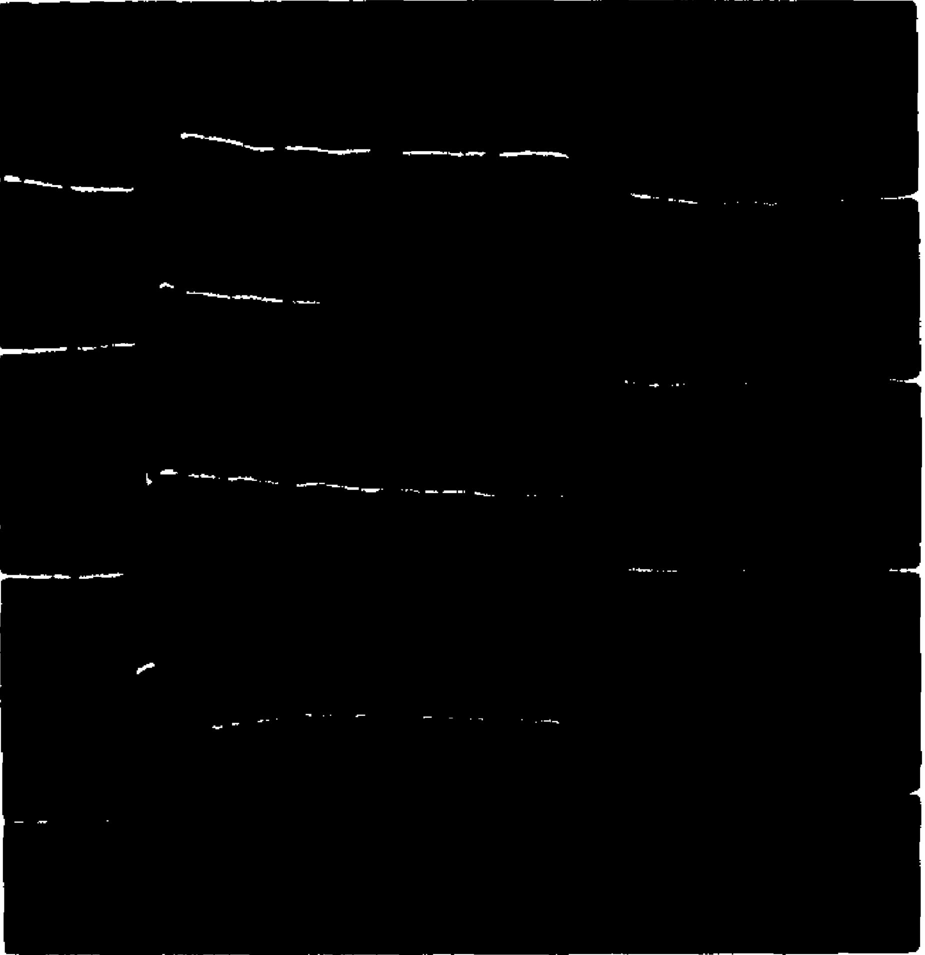
A typical intensity-response series recorded from an ocellar nerve dendrite.

Stimulus intensity increases from top to bottom.

The horizontal line at the bottom indicates duration of the stimulus.

Recorded from *Aeschna tuberculifera*.

Log I


Log I
− 3
− 2
− 1
0
10 mV
.1 sec

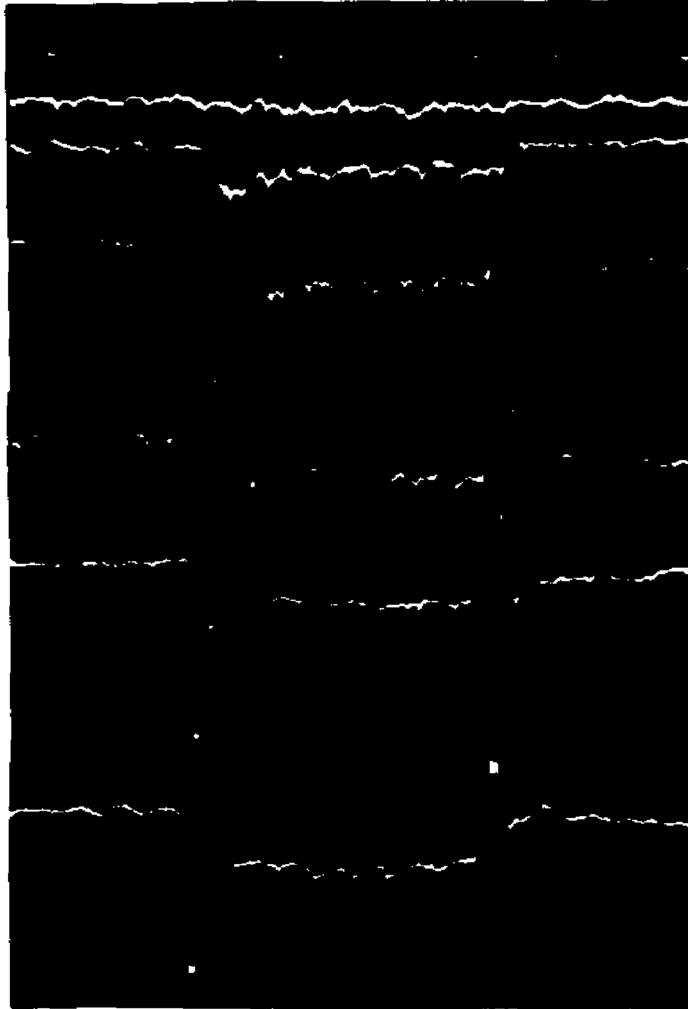

Log I
- 6
- 5
- 4
- 3
- 2
- 1
0
5 mV
.1 sec

Figure 5

An intensity-response series recorded from a receptor. At the highest stimulus intensity there is a single spike at light-on and the potential does not return to the resting level immediately after light-off.

Stimulus intensity increases from top to bottom.

The horizontal line at the bottom indicates duration of the stimulus.

Recorded from *Aeschna tuberculifera*.

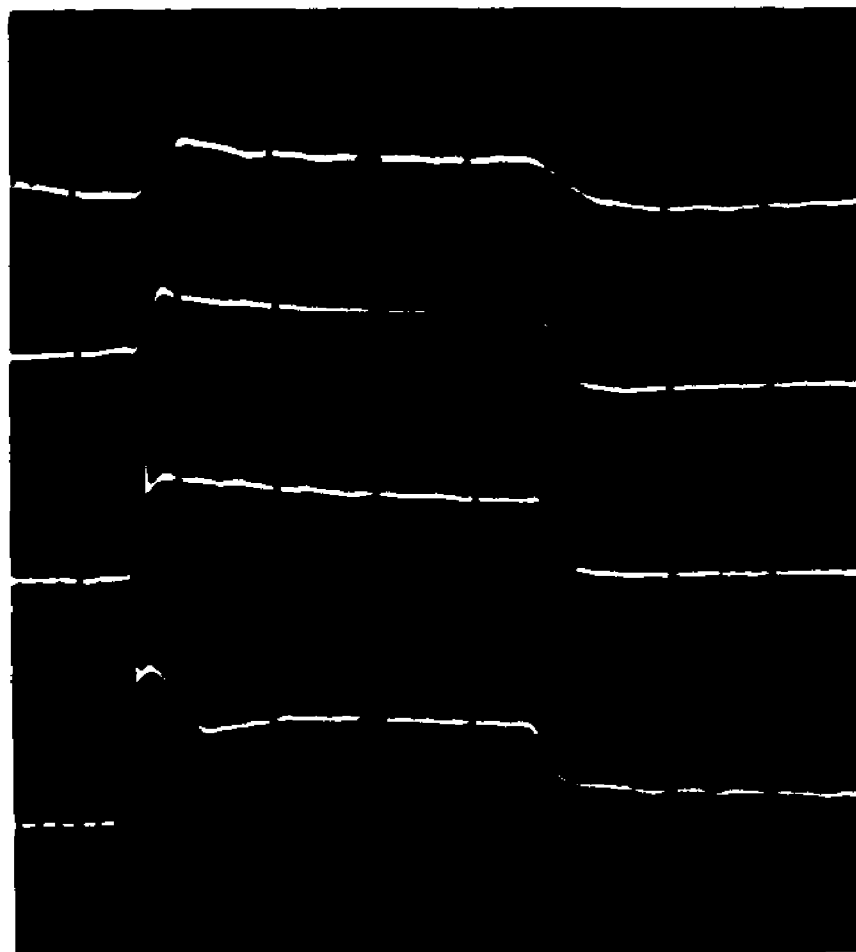

Log I
− 3
− 2
− 1
0
10 mV
.1 sec

Figure 6

Membrane resistance change of an ocellar nerve dendrite during illumination, monitored by passing 1 nAmp depolarizing pulses through the Mentor N-950 bridge system. The downward deflections mark the beginning of the pulses. The bridge potentiometer is balanced in the dark, to give a null reading of pulse amplitude. During illumination, the pulse-on portion of the trace is displaced downward, indicating that the membrane resistance has decreased. C. f. figure 2.

Log I = -3.

The horizontal line at the bottom indicates the duration of the stimulus.

Recorded from Libellula pulchella.

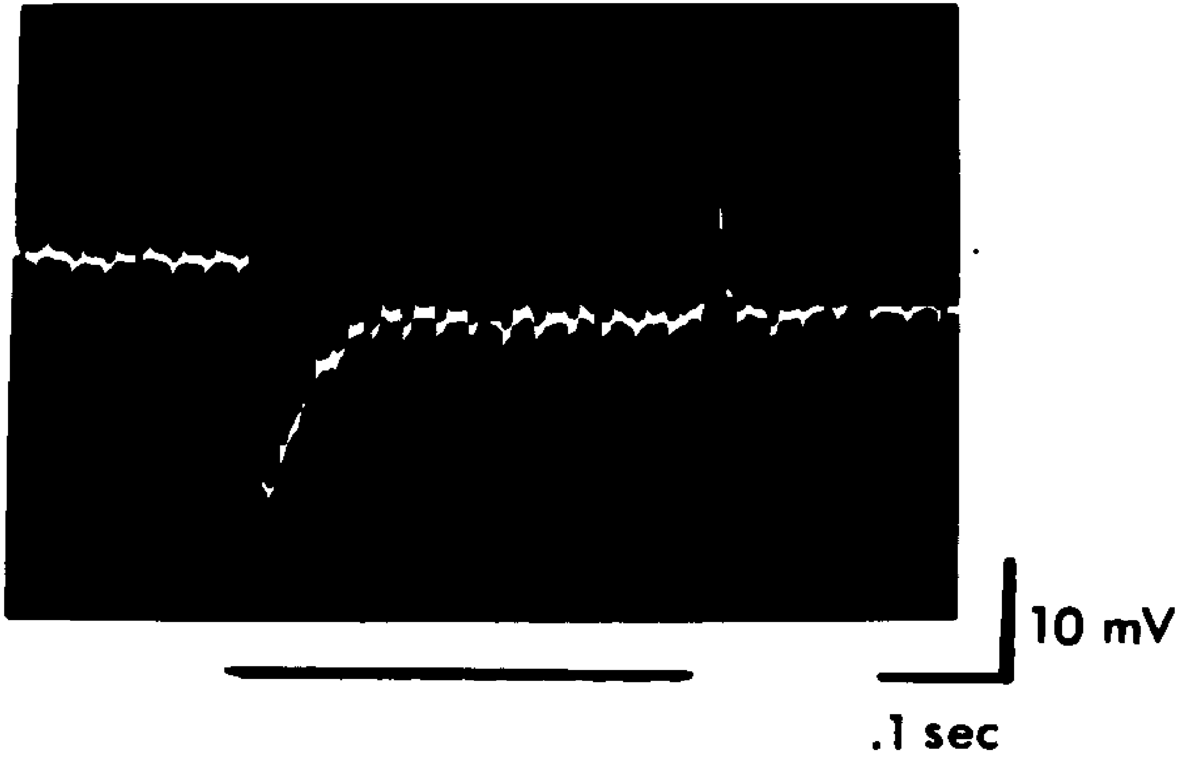

10 mV
.1 sec

Figure 7

The ocellar nerve dendrite response is reversibly blocked by 10^{-3} g/ml d-tubocurarine chloride (curare).

Log I = -2.

The horizontal line at the bottom indicates duration of the stimulus.

Recorded from *Aeschna tuberculifera*.

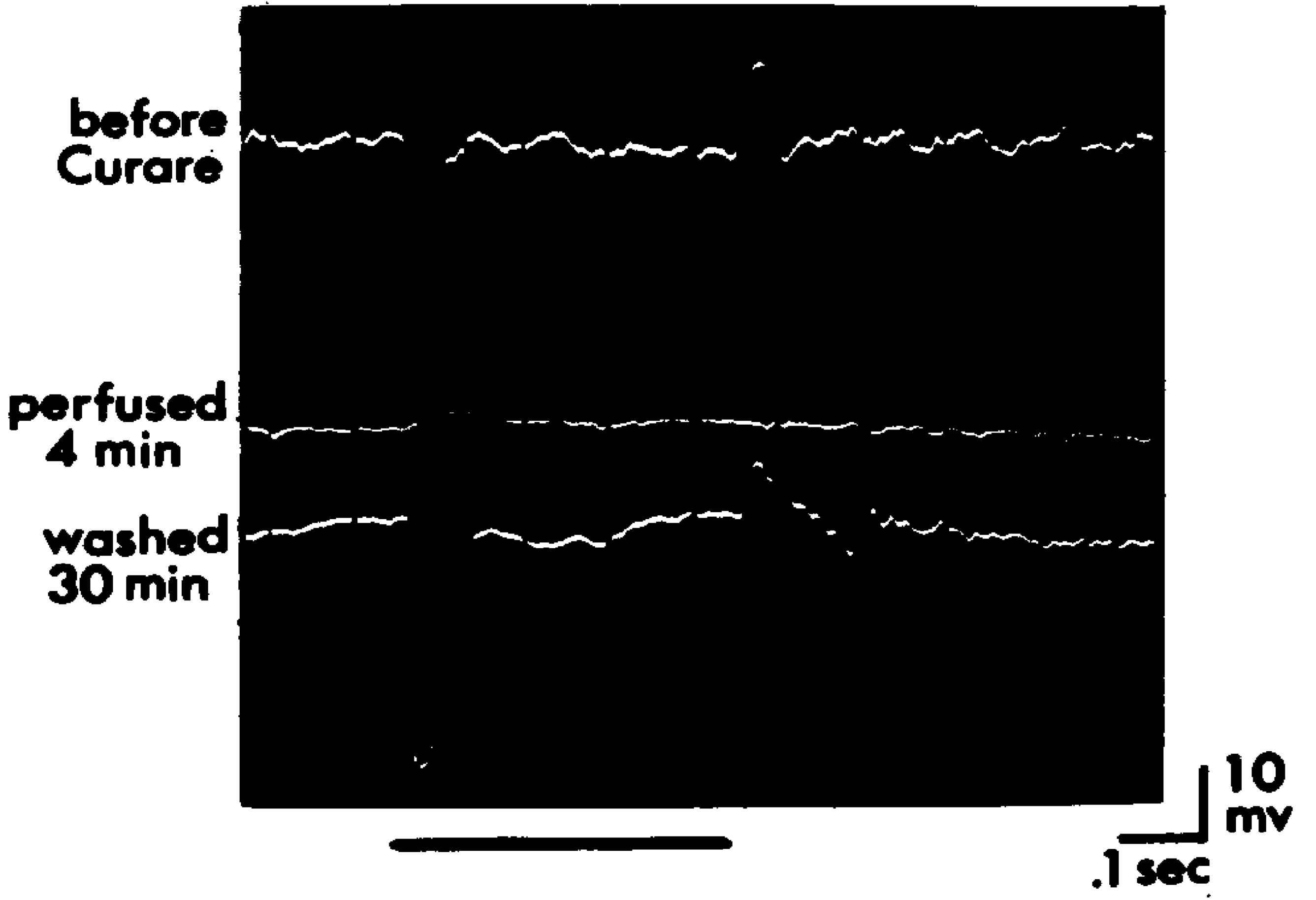

before
Curare
perfused
4 min
washed
30 min
10
mv
.1 sec

Figure 8

The ocellar nerve dendrite response is progressively decreased and finally blocked completely by perfusion with 10^{-3} g/ml curare. The resting potential did not change during this period.

Log I = -3.

The horizontal line at the bottom indicates duration of the stimulus.

Recorded from _Libellula pulchella_.

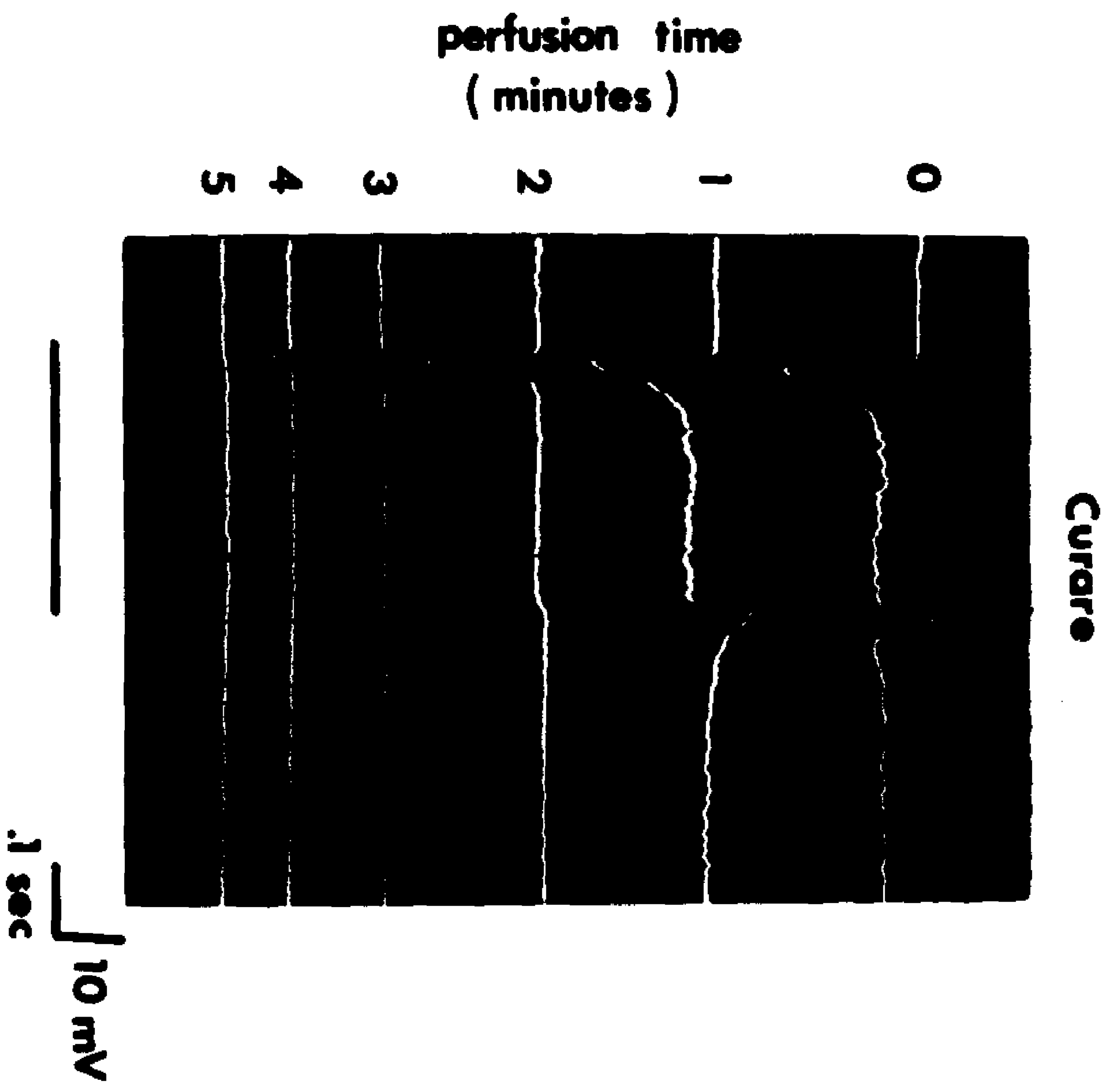

perfusion time
(minutes)
5
4
3
2
1
0
Curare
.1 sec
10 mV

Figure 9

The ocellar nerve dendrite response is reversibly blocked by 10^{-4} g/ml curare. The notch in the initial hyperpolarization was also seen in a few other units. After the light response has essentially been eliminated, a potential change is still recorded at light-on. This may represent electrical activity of the receptors. Washing with Ringer's solution restored the light response.

Log I = -1.

The horizontal line at the bottom indicates the duration of the stimulus.

Recorded from *Erythemis simplicicollis*.

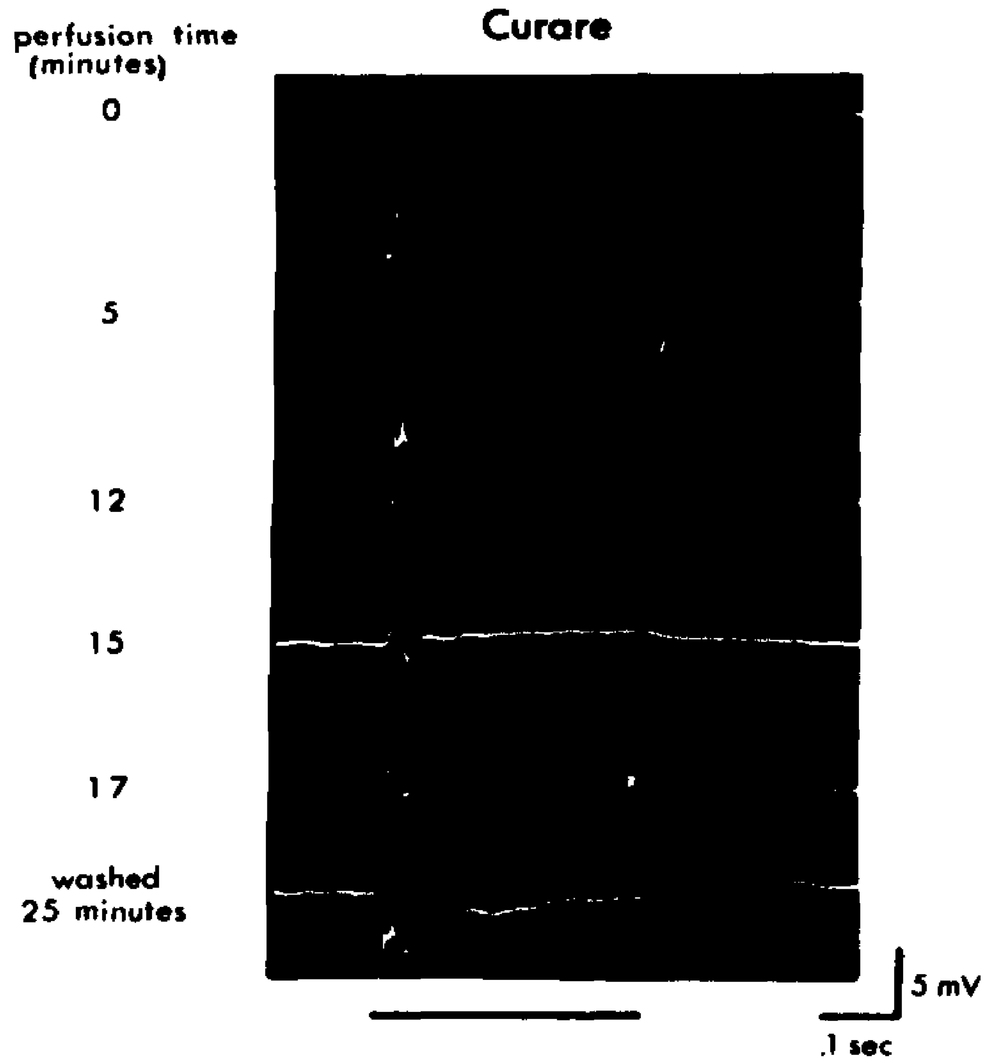

Curare
perfusion time
(minutes)
0
5
12
15
17
washed
25 minutes
5 mV
.1 sec

Figure 10

An ocellar nerve dendrite response before and after perfusion with 10^{-4} g/ml atropine sulfate. The size of the response is somewhat reduced, but there is no blocking effect comparable to that of curare (c.f. figures 6, 7 and 8).

Log I = -1.

The horizontal line at the bottom indicates the duration of the stimulus.

Recorded from *Erythemis simplicicollis*.

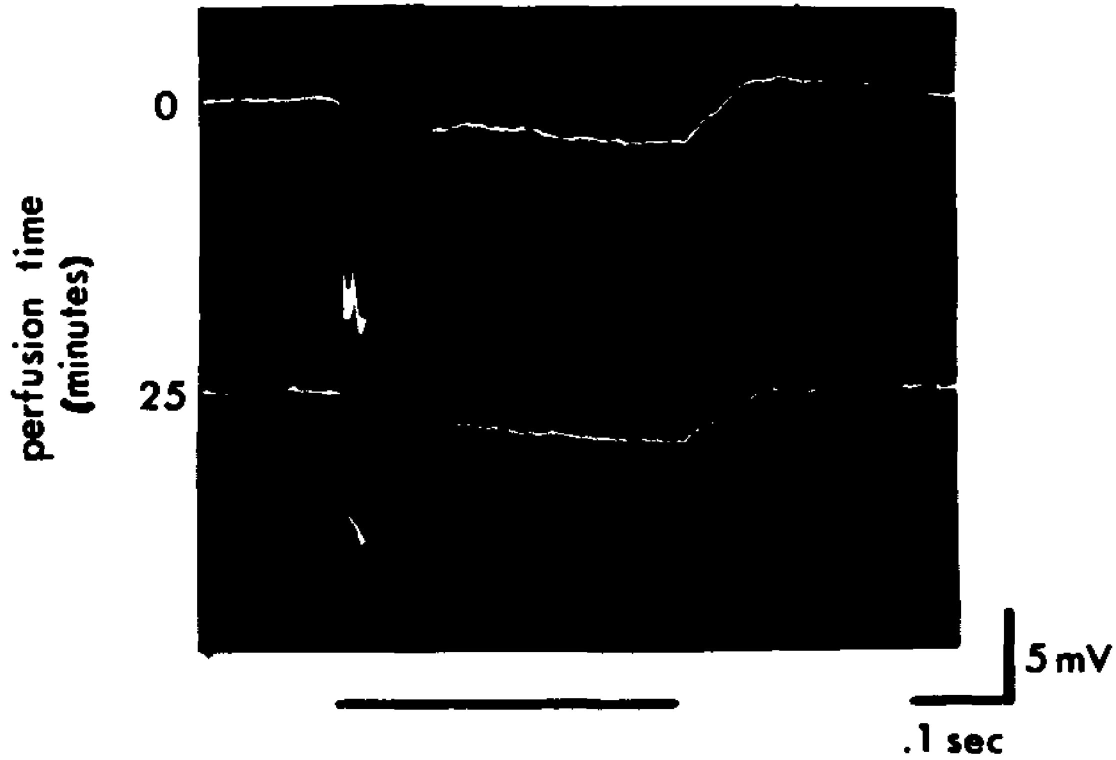

Atropine
perfusion time
(minutes)
0
25
5 mV
.1 sec

Figure 11

Intensity-response series recorded from an ocellar nerve dendrite before (left) and after (right) perfusion with 10^{-3} g/ml picrotoxin. The overshoot and oscillations after light-off are eliminated.

Stimulus intensity increases from top to bottom.

The horizontal lines at the bottom indicate duration of the stimulus.

Recorded from Plathemis lydia.

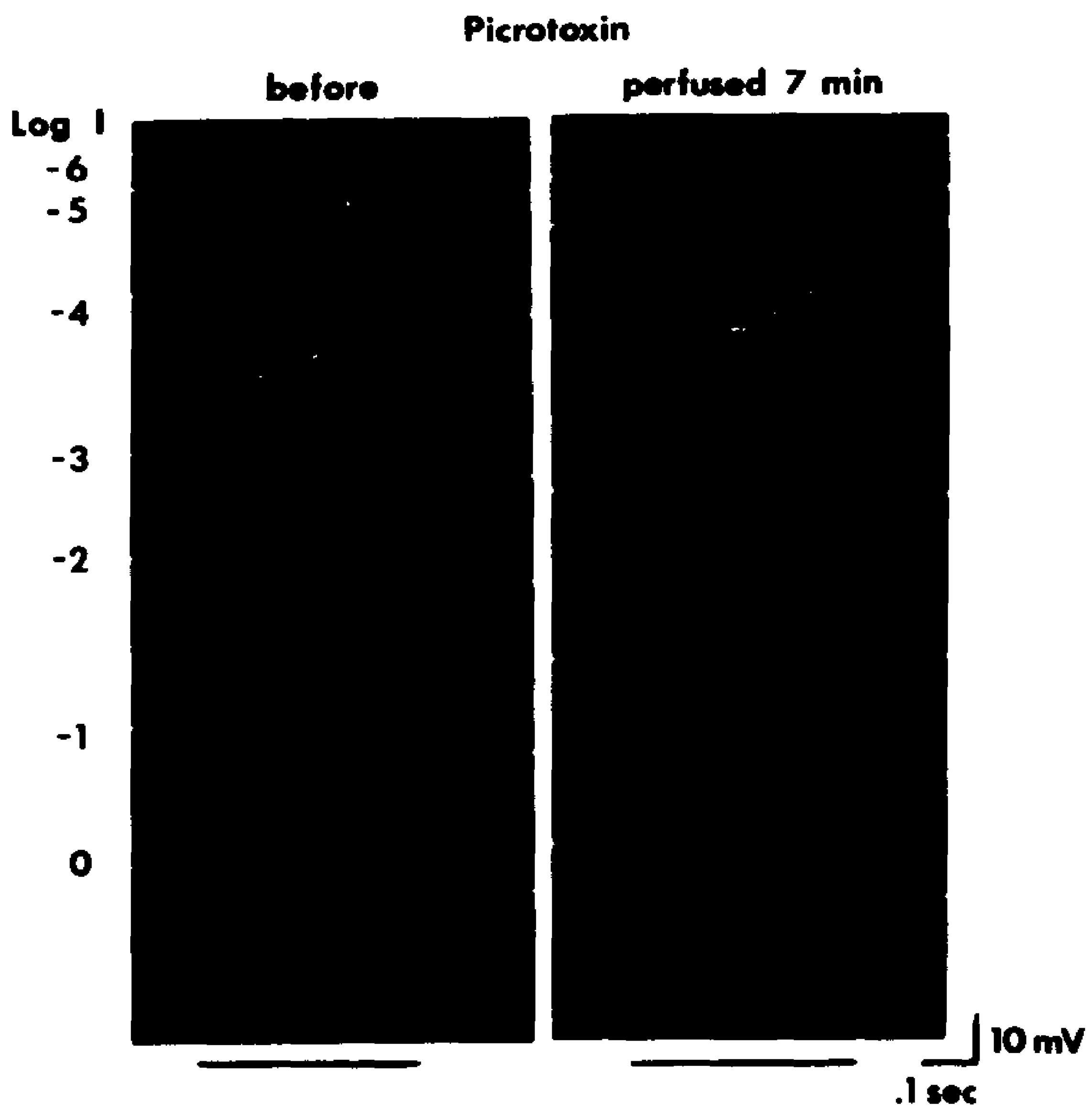

Picrotoxin
before
perfused 7 min
Log I
-6
-5
-4
-3
-2
-1
0
10 mV
.1 sec

Figure 12

The "off" response of the ocellar nerve dendrite changes in a characteristic way before disappearing, when perfused with 10^{-3} g/ml picrotoxin. The upper record shows the response before picrotoxin. In the middle record, the depolarized phase of the "off" response is increased in size and prolonged, after 2 minutes perfusion with 10^{-3} g/ml picrotoxin. In the lower record, after 4 minutes perfusion with the drug, the "off" response has disappeared completely.

Log I = -2.

The horizontal line at the bottom indicates duration of the stimulus.

Recorded from *Plathemis lydia*.

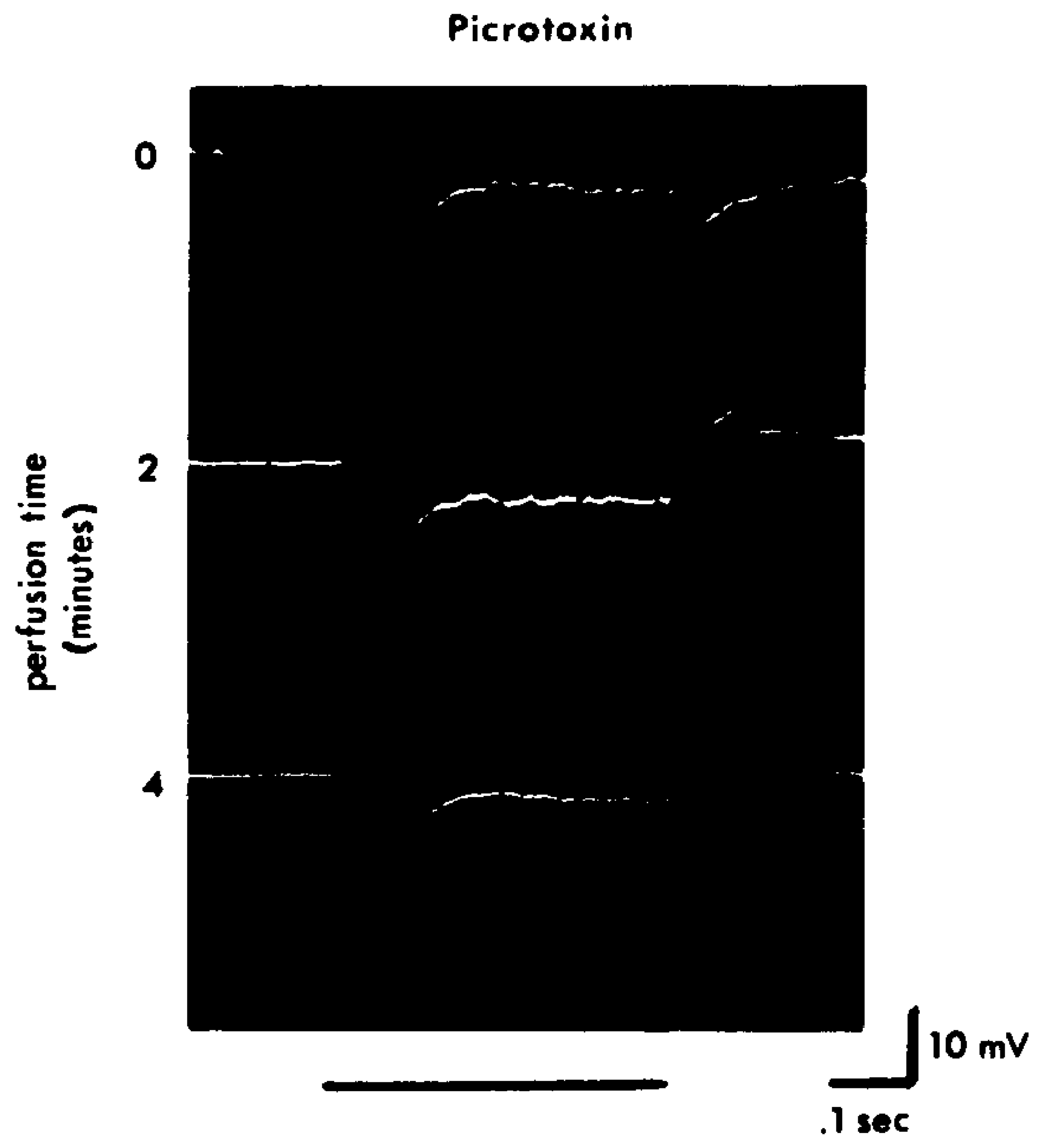

Picrotoxin
perfusion time (minutes)
0
2
4
10 mV
.1 sec

Figure 13

Intensity-response series recorded from same unit as in figure 12, washed 12 minutes with Ringer's solution after perfusion with 10^{-3} g/ml picrotoxin. The "off" response does not return to its original form, but resembles the "off" response in the middle record of figure 12, which was recorded shortly after picrotoxin perfusion was begun and before the "off" response was eliminated.

Stimulus intensity increases from top to bottom.

The horizontal line at the bottom indicates duration of the stimulus.

Recorded from *Plathemis lydia*.

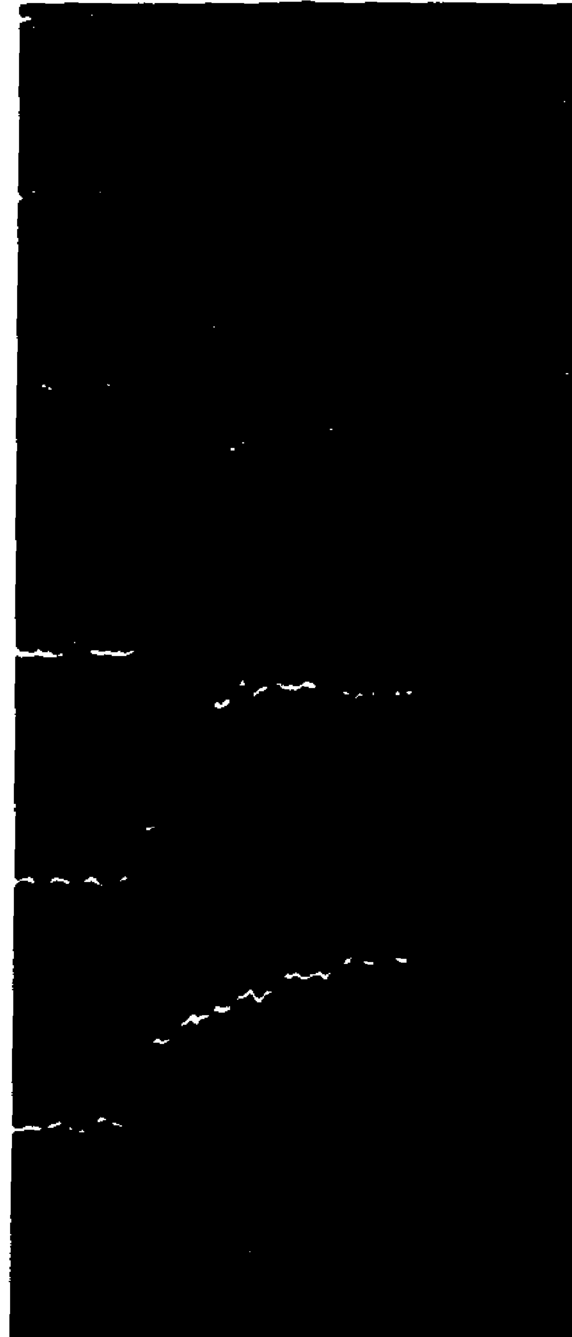

washed 12 min
after Picrotoxin
Log I
-6
-5
-4
-3
-2
-1
0
10 mV
.1 sec

Figure 14

An ocellar nerve dendrite perfuses with 10^{-5} g/ml picrotoxin. The upper record shows the response before picrotoxin. The middle record shows the response after 10 minutes perfusion with 10^{-5} g/ml picrotoxin. The "off" response has changed to a prolonged depolarization. In the bottom record, the "off" response has been restored to its phasic form after washing 40 minutes with Ringer's solution.

Log I = -2.

The horizontal line at the bottom indicates duration of the stimulus.

Recorded from _Aeschna tuberculifera_.

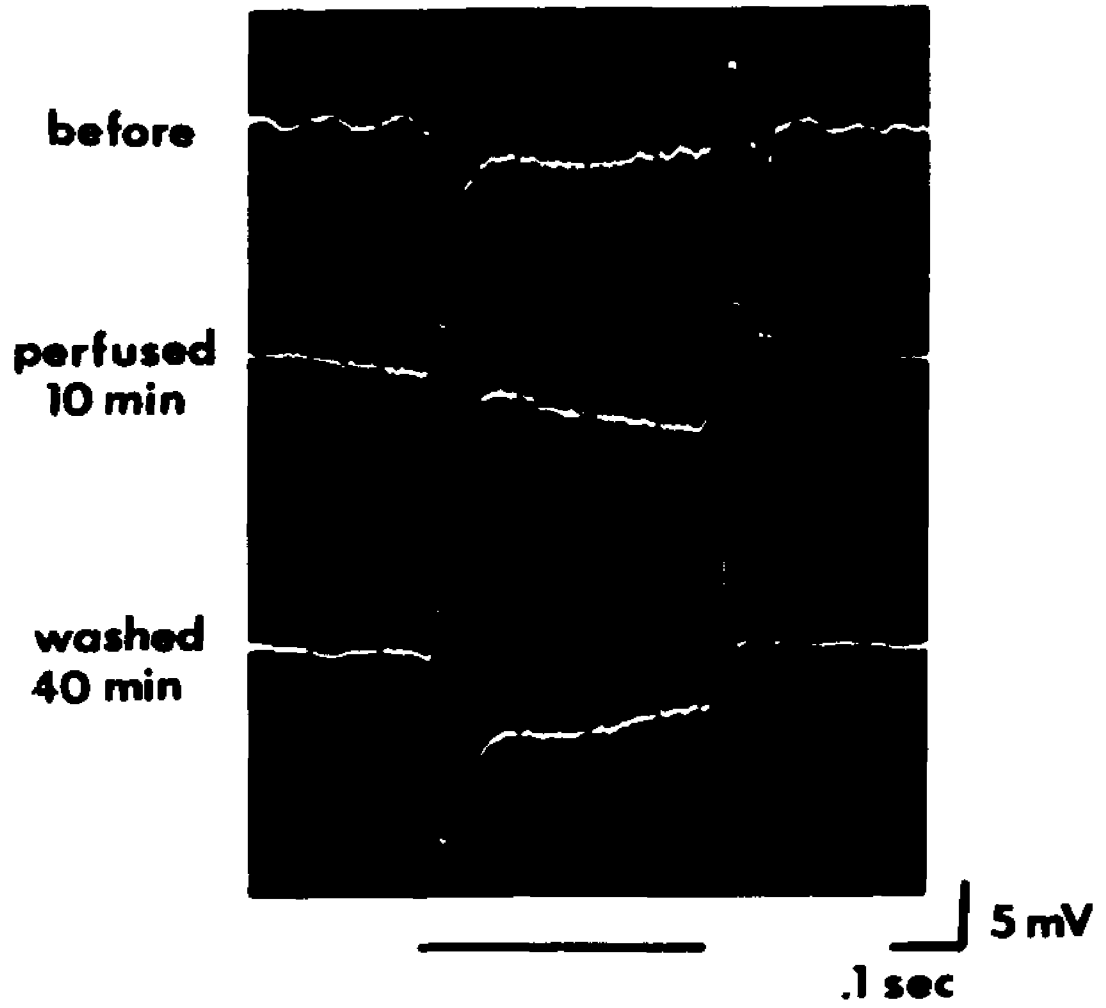

Picrotoxin
before
perfused
10 min
washed
40 min
5 mV
.1 sec

Figure 15

Three superposed responses of an ocellar nerve dendrite perfused with 10^{-5} g/ml picrotoxin. The "off" response in its altered form was the most reproducible part of the response, in spite of variations in resting potential at "on" and in the "on" response.

Log I = -2.

The horizontal line at the bottom indicates duration of the stimulus.

Recorded from *Aeschna tuberculifera*.

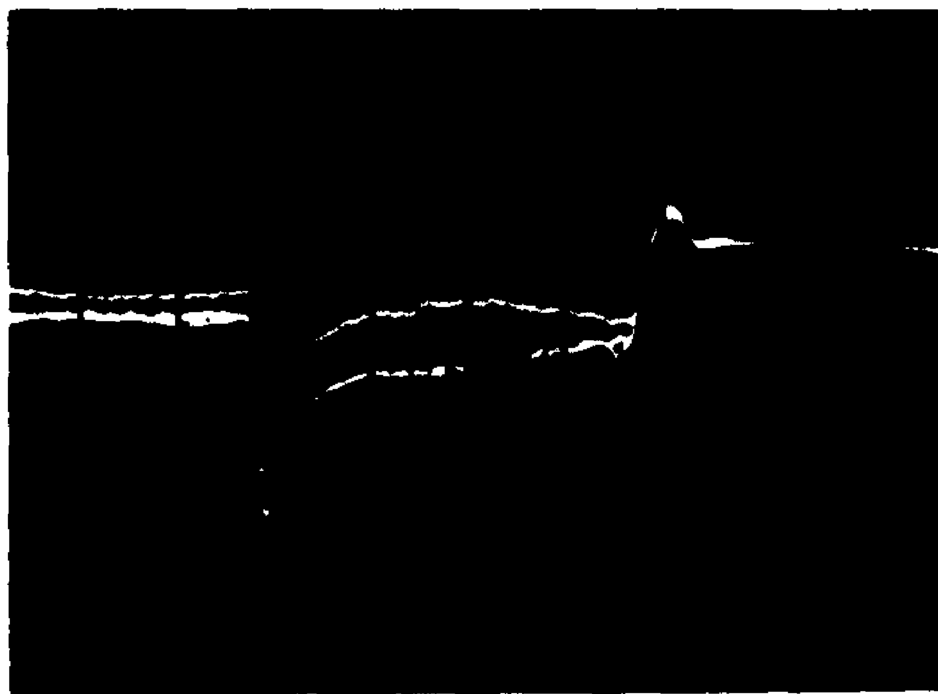

10 mV
.1 sec

Figure 16

Intensity-response series recorded from an ocellar nerve dendrite before (left) and after (right) 35 minutes perfusion with 3×10^{-5} g/ml bicuculline. After perfusion with bicuculline, the "off" overshoot is eliminated, the noise in the dark is reduced, the "on" transient is smaller, and the potential just before the end of the 400 msec light flash is smaller, except at the highest intensities.

Stimulus intensity increases from top to bottom.

The horizontal lines at the bottom indicate duration of the stimulus.

Recorded from *Aeschna tuberculifera*.

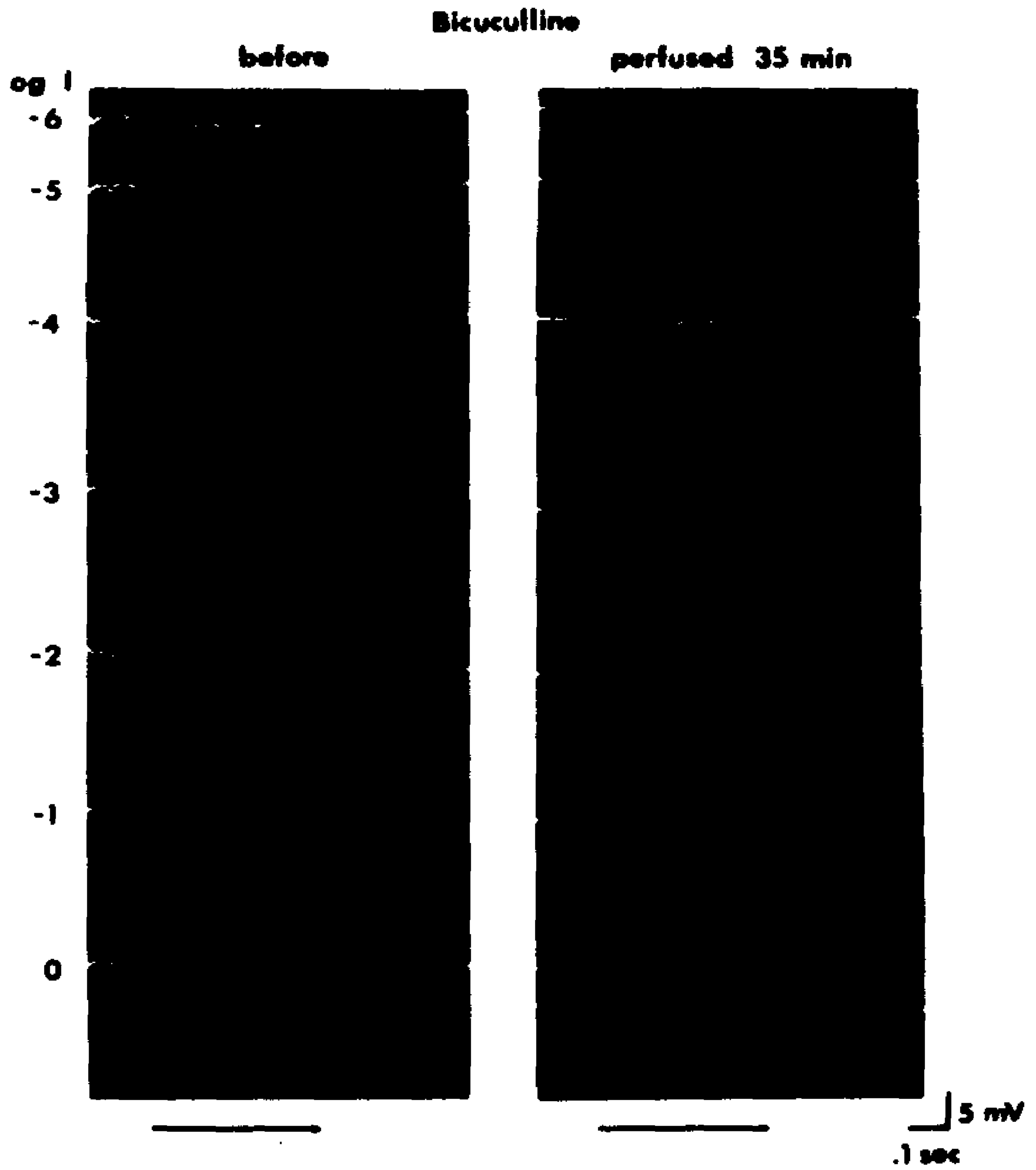

Bicuculline
before
perfused 35 min
og I
-6
-5
-4
-3
-2
-1
0
5 mV
.1 sec

Figure 17

Intensity-response series recorded from an ocellar nerve dendrite before (left) and after (right) 15 minutes perfusion with 10^{-4} g/ml bicuculline. The "off" overshoot was greatly reduced. At this point, washing with Ringer's solution was begun rather than continuing perfusion with bicuculline, but the unit was lost shortly after beginning the wash.

Stimulus intensity increases from top to bottom.

The horizontal lines at the bottom indicate duration of the stimulus.

Recorded from *Aeschna tuberculifera*.

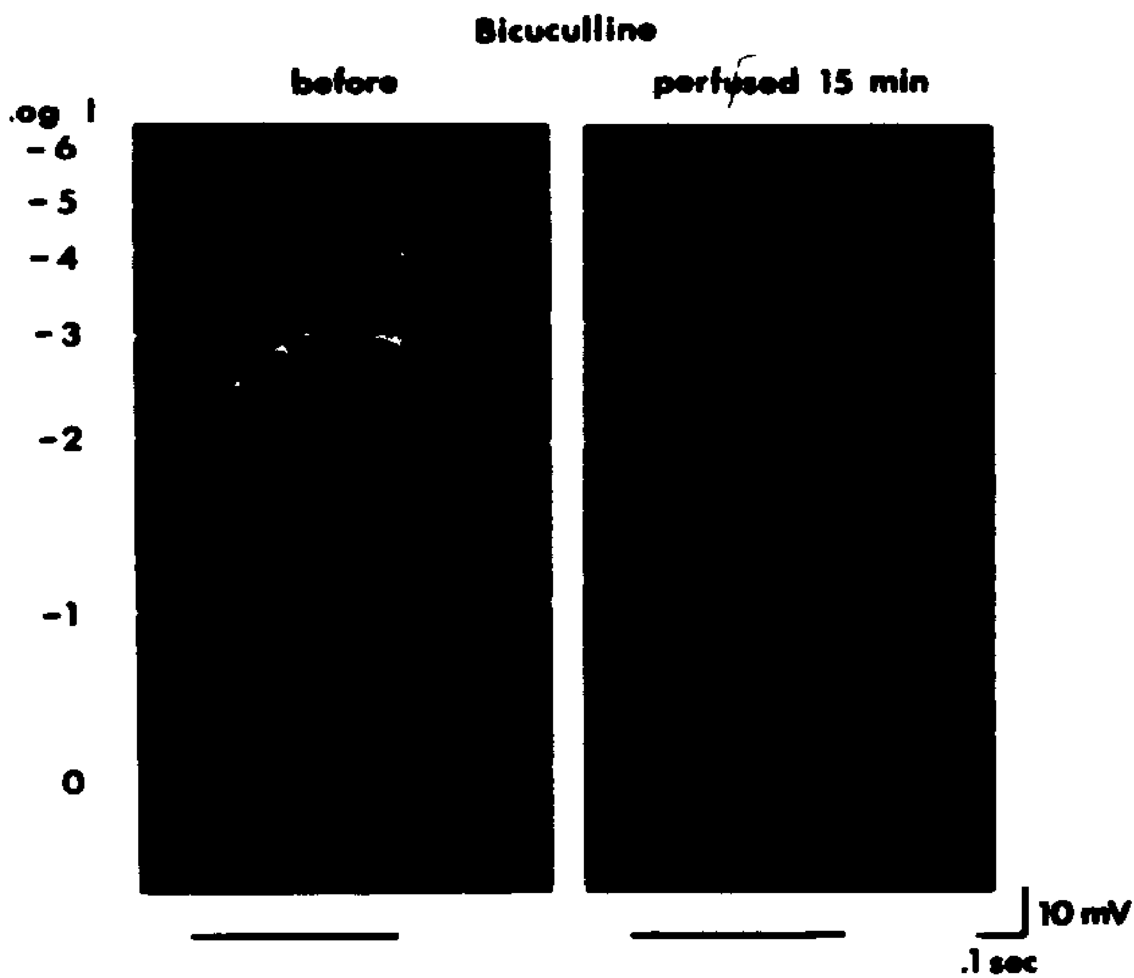

Bicuculline
before
perfused 15 min
log I
-6
-5
-4
-3
-2
-1
0
10 mV
.1 sec

Figure 18

An ocellar nerve dendrite perfused with a mixture of 10^{-4} g/ml acetylcholine and 10^{-5} g/ml edrophonium, an anti-cholinesterase drug. The number at the beginning of each record is the resting potential at that time. The resting potential became progressively more negative after perfusion with the drugs was begun, with the exception of one brief fluctuation in the positive direction, shown in the next to last record. At the same time the size of the light response decreased.

Log I = -3.

The horizontal line at the bottom indicates duration of the stimulus.

Recorded from *Aeschna tuberculifera*.

ACh + Edrophonium

perfusion time
(minutes)

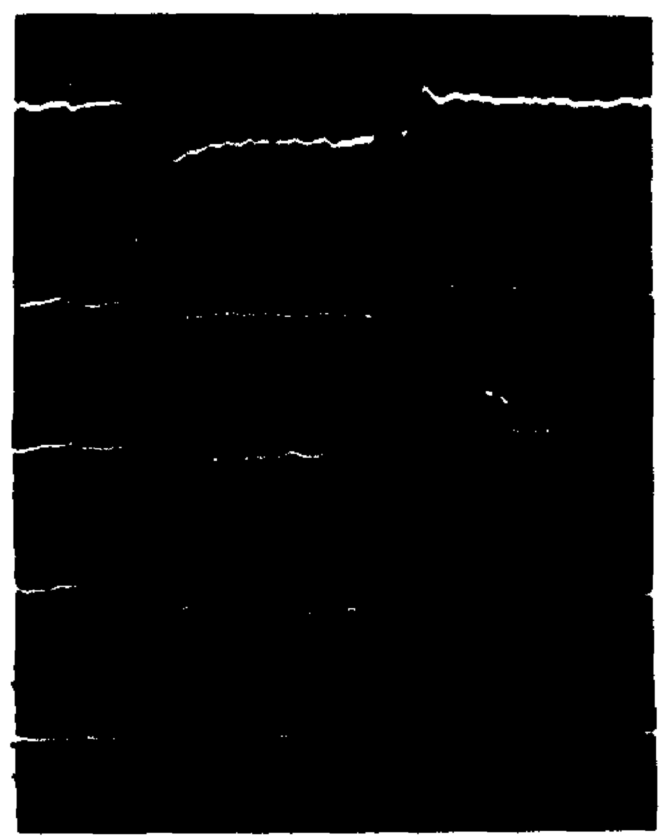
0

10

10.5

11

12



10 mV

.1 sec

Figure 19

An ocellar nerve dendrite perfused with a mixture of 10^{-4} g/ml acetylcholine, 10^{-5} g/ml edrophonium and 10^{-3} g/ml curare. There is no hyperpolarization in the dark as occurred with acetylcholine and edrophonium, and the response to light is eliminated. Curare appears to block both the hyperpolarizing effect of applied acetylcholine and edrophonium (c.f. figure 17) and the response to light.

Log I = -2.

The horizontal line at the bottom indicates duration of the stimulus.

Recorded from *Aeschna tuberculifera*.

ACh + Edrophonium + Curare

perfusion time
(minutes)

0

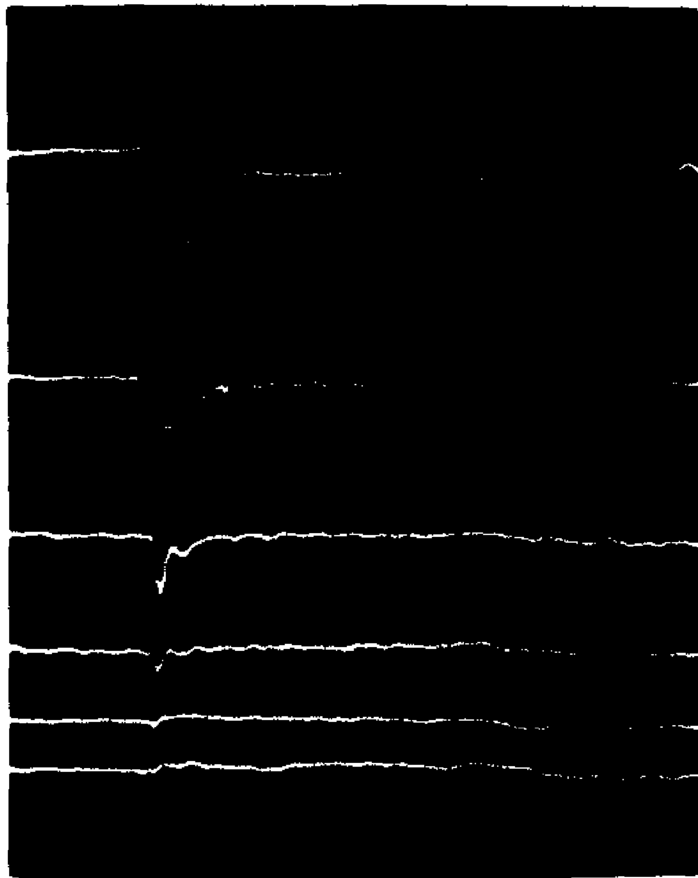
3

6

8

11

15



10 mV

.1 sec

Figure 20

Intensity-response series recorded from an ocellar nerve dendrite before (left) and after (right) 23 minutes perfusion with 10^{-3} g/ml GABA. There is an increase in the size of the "on" response and a change in the form of the "off" response.

Stimulus intensity increases from top to bottom.

The horizontal lines at the bottom indicate duration of the stimulus.

Recorded from *Aeschna tuberculifera*.

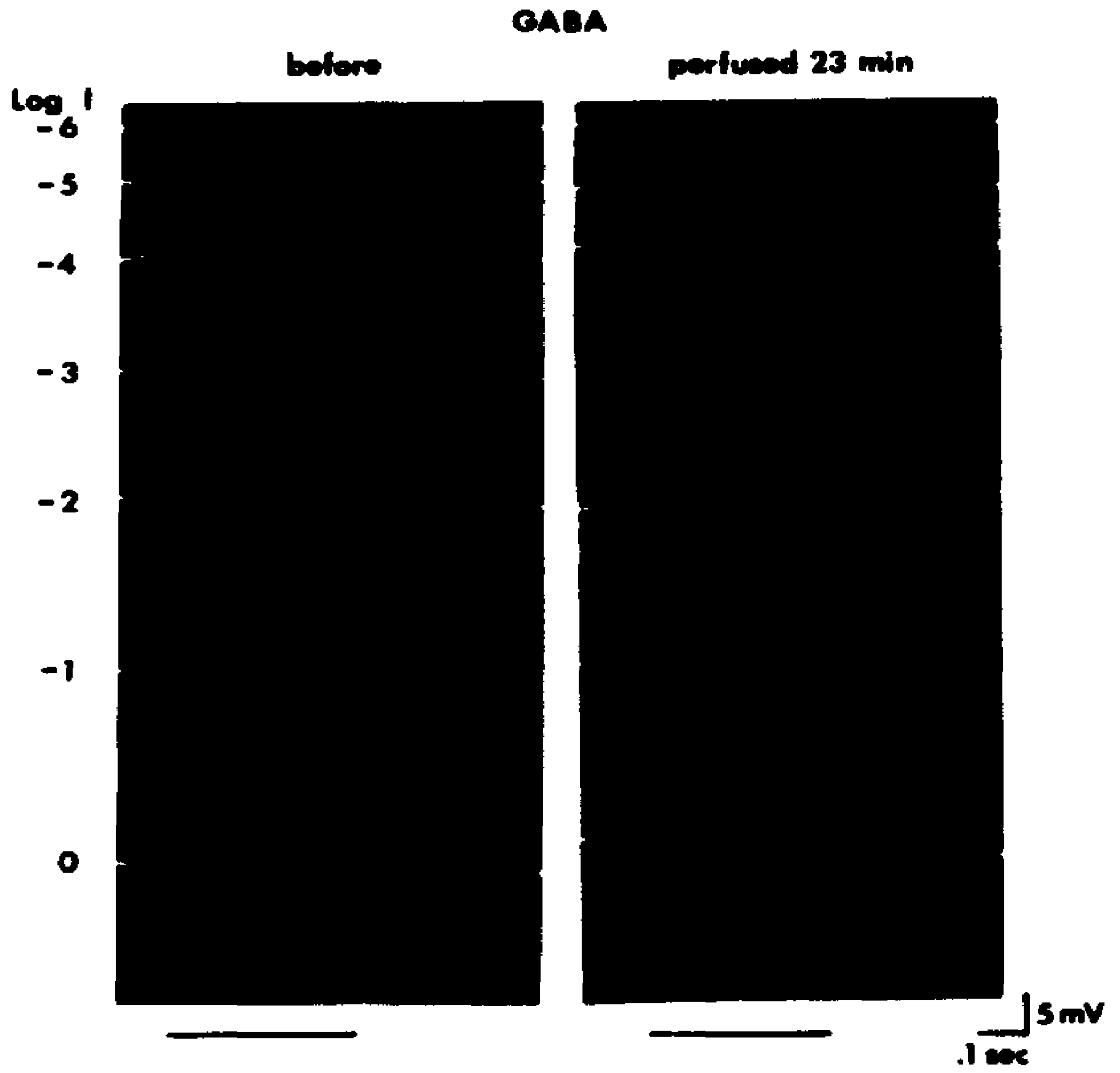

GABA
before
perfused 23 min
Log I
-6
-5
-4
-3
-2
-1
0
5 mV
.1 sec

Figure 21

Responses recorded from the same ocellar nerve dendrite as in figure 20, before GABA, after 23 minutes perfusion with 10^{-3} g/ml GABA, and after washing with Ringer's solution. Shortly after washing was begun, the "off" response regained its oscillatory character. After 6 minutes of washing the unit was lost.

Log I = -2.

The horizontal line at the bottom indicates duration of the stimulus.

Recorded from *Aeschna tuberculifera*.

GABA

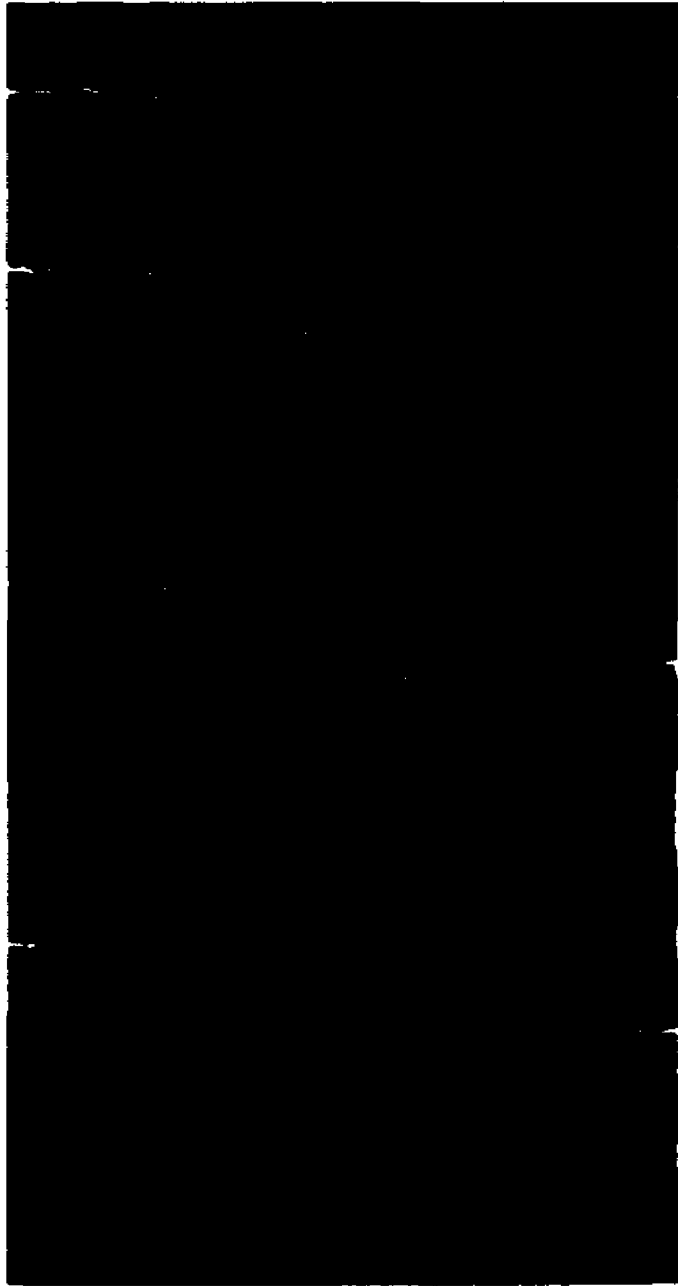


Figure 22

Schematic model of facilitory feedba~k loop showing how such a system could account for the typical features of the ocellar nerve dendrite response. RT is the receptor terminal and OND is the ocellar nerve dendrite. The arrows represent amounts of transmitter relative to the dark equilibrium condition and do not indicate actual amounts of transmitter.

The figures from left to right show a progression from the dark adapted condition through a light stimulus and back to darkness again. The tracing at the bottom represents the potential changes recorded in the ocellar nerve dendrite during this period. See text for further details.

(A) dark adapted

(B) light on

(C)

(D) steady state

(E) light off

(F)

(G) dark adapted

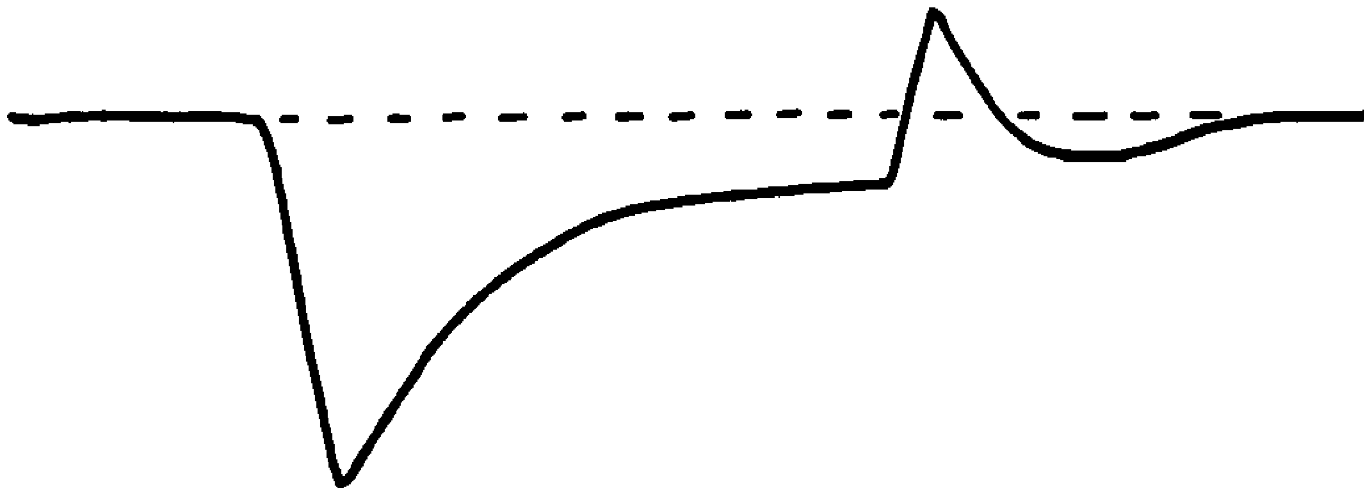
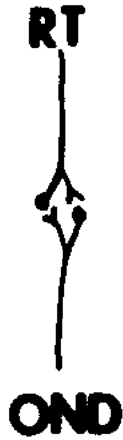


Figure 23

The effect of bicuculline on the facilitory feedback loop presented in figure 22. RT is the receptor terminal and OND is the ocellar nerve dendrite. The arrows represent relative amounts of receptor transmitter. The feedback transmitter is assumed to be blocked by bicuculline. The tracing at the bottom represents the potential changes recorded in the bicuculline-treated ocellar nerve dendrite when a light stimulus is turned on and then off. See text for further details.

(A)
dark
adapted



(B)
light
on

(C)
steady
state

(D)
light
off

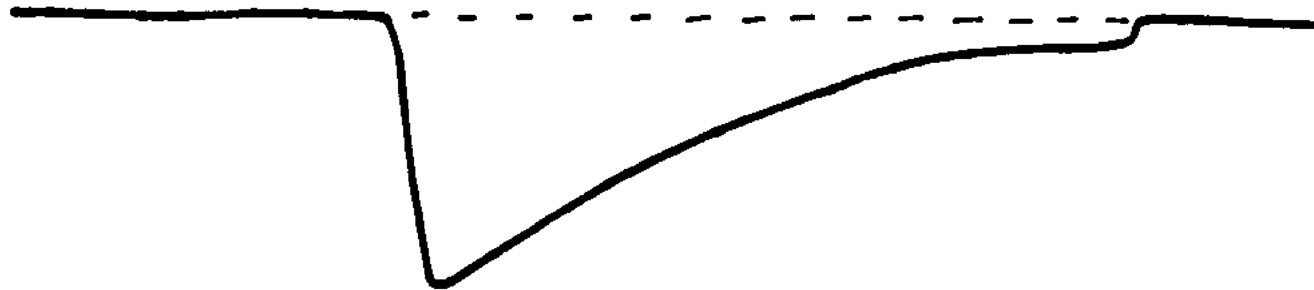


Figure 24

Potentials predicted by the facilitory feedback model in the normal and bicuculline-treated cases. The solid line represents the normal situation and the broken line represents the bicuculline-treated ocellar nerve dendrite. See text for further details.

APPENDIX

On the assumption that feedback from ocellar nerve dendrites to receptor terminals modifies the light response in the latter and thus modifies transmission from receptors to ocellar nerve dendrites, I attempted to develop a receptor preparation in which receptor responses unmodified by feedback from ocellar nerve dendrites could be recorded. This was to be done by severing the ocellar nerve in the nymph. I had hoped that the ocellar nerve fibers would degenerate but that the receptors would continue to develop and that after metamorphosis the ocelli of the adult dragonflies would have receptor cells that appeared normal except that they would not be innervated by ocellar nerve fibers.

Dragonfly nymphs purchased from our supplier and raised in the laboratory were received at various stages of nymphal development and some went through as many as three or four instars before reaching the adult stage. From preliminary dissections of nymphs of different sizes I found that ocellar receptors and nerve fibers were visible in the larger nymphs but not in the smaller ones. I then began a study to determine when the ocellar structures first appeared and when and how the ocellar nerve could be severed. Since almost all of the nymphs raised in the laboratory proved

upon metamorphosis to belong to one species, *Aescha tuberculifera*, problems due to species differences in development were not expected.

There is no literature available on developmental stages of the nymph of *Aeschna tuberculifera*. A study of nymphal development of *Anax junius*, a closely related species, by Calvert (1934) describes 13 larval stages, their distinguishing features, and the duration of each instar, and stresses the variability of appearance of the nymph and length of instars among different individuals.

With no developmental literature to draw on, it was first necessary to learn to judge the age of nymphs by examination of external features so that I could select for the nerve-severing operations nymphs which were at the proper stage of development. For this purpose I selected a total population of 32 numphs of various sizes. I first attempted to assign them to matched groups on the basis of length and weight. I rejected weight as very unreliable because nymphs take in and expel water and consequently their weight varies by as much as a factor of two. However, the variation in length due to this behavior is at most 10%. The nymphs were measured weekly and also on the day following each moult. Within the matched groups, some of the nymphs (12 in all) were dissected at various stages. To look for

ocellar structures in these nymphs, the head was removed, cut into dorsal and ventral halves, and examined under a dissecting microscope. Other nymphs (7 in all) were allowed to metamorphose into adults as controls for accurately determining the age of the dissected nymphs, and all seven proved to be *Aeschna tuberculifera*. Eight nymphs died of natural causes. Weight and length data were taken on another 5 nymphs, which were not followed through to metamorphosis or death before the study was completed. The following results were obtained:

Instar	Length of nymph on 2nd day of instar	
	Range	Mean ± S.D.
final	32 - 39 mm	35.88 ± 3.09
pre-final	26 - 32 mm	29.18 ± 4.33
pre-pre-final	19 - 25 mm	22.80 ± 5.76

It can be seen that there is some overlap of the range of lengths measured at the beginning of the final instar and of the instar preceding the final one. However, a nymph which has just entered an instar and measures around 32 mm can still be unambiguously classified by the degree of separation of the wing case from the body, the separation being distinctly greater in the final instar nymphs.

Ocellar structures were examined in the 12 nymphs from the above group which were dissected, and in another 15 nymphs in the final and the pre-final instars. From day 9 of the final instar, structures very similar in appearance to the adult median and lateral ocelli are found lying under the three black markings on the vertex, on the dorsal surface of the head. There are well-formed, milky-white receptor-like structures, and fibers running between these and the ganglia which form the protocerebrum in the adult. Layers of brown pigment, similar to those which surround the adult ocelli, are also present. Since this study was completed, rhabdomes have been seen in light micrographs of the ocellar receptor organs of dragonfly nymphs in the late final instar (Katz & Chappell, 1975).

Up to the fourth day of the final instar, less distinct fibers can be seen running between the ganglia and the same location on the inside of the dorsal exoskeleton, but no receptor-like structures are visible under the dissecting microscope. In some nymphs there is a slight mass at the distal end of these fibers, but in others it is not clear to what the fibers are attached distally. Between the fourth and ninth day of the final instar the ocellar receptor organ and the fibers which connect it to the ganglia become clearly visible.

Nymphs dissected in the second half of the pre-final instar are similar to those examined during the first four days of the final instar. In nymphs in the first half of the pre-final instar nothing could be seen in the ocellar region by this method.

Operations to sever the ocellar nerve were attempted in 22 nymphs which were in the second and third week of the final instar. In many of these, the ocellar receptors could be seen through the exoskeleton. From earlier dissections, I knew that the ocellar nerve at this stage was closely packed between the protocerebrum and the ocellar receptor organ which lay directly above it. The first step in the operation was to remove a small piece of exoskeleton from the vertex posterior to the median ocellus. This piece of exoskeleton was placed in Ringer solution and saved for later use. The exposed tissue was also kept moist with Ringer solution. A Weck microscissors with curved blades was used to make a shallow cut under the median ocellar receptor from the posterior end. The cut was made in a plane parallel to the top of the head. By this time the tissue was usually protruding somewhat through the opening in the exoskeleton. The piece of exoskeleton was replaced and sealed on with Tackiwax, and the nymph was returned to the tank. Most of the operated nymphs began to swim in an

apparently normal manner when returned to the tanks, but a few remained inactive for several minutes or hours. However, serious damage must have occurred, since only 2 of the 22 nymphs survived through to the adult stage. Of these two adults, one was dissected and the ocellar nerve was found uncut. The other was lost due to a mistake in labeling. Of the remaining 20, 13 died before reaching metamorphosis and 5 died during metamorphosis. All of these were dissected, and in only one the ocellar nerve was found to be cut while the receptor organ appeared to be intact. In the others, there were varying degrees of damage to surrounding tissues, particularly the ocellar receptor organ and lens, and in most cases the damage was so extensive that it was not clear whether the ocellar nerve had actually been cut. The remaining two of the operated nymphs were lost before metamorphosis. Because of the poor results described here, I concluded that this approach was not feasible.

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