Feedback Synaptic Interaction in the Dragonfly Ocellar Retina

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ABSTRACT The intracellular response of the ocellar nerve dendrite, the second order neuron in the retina of the dragonfly ocellus, has been modified by application of various drugs and a model developed to explain certain features of that response. Curare blocked the response completely. Both picrotoxin and bicuculline eliminated the "off" overshoot. Bicuculline also decreased the size of response and the sensitivity. γ-Aminobutyric acid (GABA), however, increased the size of response. The evidence indicates the possibility that the receptor transmitter is acetylcholine and is inhibitory to the ocellar nerve dendrite whereas the feedback transmitter from the ocellar nerve dendrite may be GABA and is facilitory to receptor transmitter release. The model of synaptic feedback interaction developed to be consistent with these results has certain important features. It suggests that the feedback transmitter is released in the dark to increase input sensitivity from receptors in response to dim light. This implies that the dark potential of the ocellar nerve dendrite may be determined by a dynamic equilibrium established by synaptic interaction between it and the receptor terminals. Such a system is also well suited to signalling phasic information about changes in level of illumination over a wide range of intensities, a characteristic which appears to be a significant feature of the dragonfly median ocellar response.

INTRODUCTION

Both anatomical and electrophysiological evidence for synaptic feedback in the retina of the dragonfly median ocellus is now known. Anatomically, one finds reciprocal synaptic arrangements between the receptors and second order cells, the ocellar nerve dendrites (ONDs). Both receptors and ONDs make button synapses with postsynaptic dyads (Dowling and Chappell, 1972) similar to the ribbon synapses of vertebrate bipolar cells (Kidd, 1962; Dowling and Boycott, 1966). Electrophysiological studies show the receptors to give a sustained depolarizing response to light, whereas ONDs give a hyperpolarizing response which is much more phasic and characterized by an "off" overshoot associated with a burst of impulses (Chappell and Dowling, 1972).

The relative simplicity of the dragonfly ocellar retina makes the preparation well suited to a study of synaptic transmission, there being just two basic types of neurons: receptor cells and ONDs which go to the brain (Cajal, 1918; Ruck and Edwards, 1964). In this report, we present results of efforts to dissect

pharmacologically the direct and feedback synapses between these two cell types and discuss their relative contributions to the generation of the response recorded from ONDs in terms of a model consistent with the results.

MATERIALS AND METHODS

Preparation

Experiments were performed on dragonflies captured in the wild or laboratory-reared from nymphs supplied by Connecticut Valley Biological Supply Co., Southhampton, Mass. Recordings were made from five species of dragonfly: *Aeschna tuberculifera*, *Libellula pulchella*, *Plathemis lydia*, *Erythemis simplicicollis*, and *Anax junius*. Receptor and OND responses were similar in all species used, whether wild or laboratory-reared (Klingman, 1976).

Perfusion

Before recordings were made, dragonfly heads were removed from the body, and part of the exoskeleton was dissected from the top of the head to expose the receptor structure and ocellar nerve *in situ* (Chappell and Dowling, 1972). Ringer solution and solutions containing drugs were applied by drop perfusion from 3-inch-long, 30-gauge needles which were connected by PE 90 Intramedic tubing to 10-ml syringes fitted with Luer-Lok valves which could be opened or closed during recording to deliver small droplets at a rate of about 1 ml in 20 min. A meniscus formed in the head over the ocellus and brain while solutions drained out through the bottom of the head as drops were added to maintain a continuous perfusion.

The Ringer solution used had the following composition: 134 mM NaCl, 5.4 mM KCl, 3.8 mM $CaCl_2$, 3.0 mM $MgCl_2$, 0.5 mM $NaHCO_3$; pH 7.4. Ionic composition of the Ringer was based on analysis of *Aeschna* nymph hemolymph (Duchateau et al., 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., Stamford, Conn.; atropine sulfate and *d*-tubocurarine chloride, Mann Research Laboratories, New York; bicuculline, K & K Laboratories, Inc., Plainview, N. Y., and Pierce Chemical Co., St. Louis, Mo.; edrophonium chloride, Hoffmann-La Roche, Inc., Nutley, N. J.; gamma-amino-*n*-butyric acid and picrotoxin, Sigma Chemical Co., St. Louis, Mo.

Recordings

Recordings were made using 50–100 megohm fiber-filled electrodes, a Mentor N-950 intracellular probe system (Mentor Corp., Minneapolis, Minn.) and a Tektronix 502 oscilloscope (Tektronix, Inc., Beaverton, Ore.) whose output was recorded on a Vetter model A FM tape recorder (A. R. Vetter Co., Rebersburg, Pa.). Membrane resistance changes during illumination were observed by passing 1-nA depolarizing pulses at a frequency of 10 pulses/second with either 10 or 20% duty cycle through the Mentor N-950 bridge system with bridge balance adjusted to null pulse amplitudes in the dark.

Photostimulator

A 100W Osram quartz-iodide bulb controlled by an 8.3 A DC constant current source was used to illuminate the ocellus. The beam was brought to focus into one end of a 3-mm 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 determined to be 675 footcandles using an optometer model 40X light meter (United Detector Technology Inc., Santa Moncia, Calif.) traceable to the National Bureau of Standards. This intensity is referred to as log I = 0, with lower intensities (referred to as log I = −1, etc.) produced using Kodak Wratten neutral density filters (Eastman Kodak Co., Rochester, N. Y.) to attenuate the beam.

RESULTS

Responses similar to the hyperpolarizing responses to light previously reported for ONDs (Chappell and Dowling, 1972) were recorded from all five species of dragonfly used in this study. Responses stable enough for pharmacological investigations were obtained in about 10% of the preparations. Resting potentials generally ranged from −35 to −55 mV, inside negative. In one unit, evidence of impulse activity was seen during intracellular recording; otherwise, impulses were not recorded as part of the OND responses, similar to the experience of Chappell and Dowling (1972).

A typical intensity-response series is shown on the left of Fig. 4. Variations from cell to cell may be observed by comparison with t = 0 responses in Figs. 1, 2, and 3. 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 and −25 mV. The amplitude did not appear to be related to the size of the resting potential, and the variations seen may have been a function of the recording site within the OND or the uneven illumination of the receptor populations feeding the ONDs. Compared with the responses shown by Chappell and Dowling (1972), the off responses recorded in this study were generally more oscillatory, with both overshoots and undershoots of the resting potential, rather than returning directly to the resting potential after a more prolonged depolarization. Change in cell membrane resistance in response to illumination was carefully evaluated in recordings from three ONDs. In each case, the balanced impedance bridge became unbalanced during illumination in the direction indicating a decrease in resistance of the OND membrane during illumination (Klingman, 1976).

Pharmacological studies of the effects of drugs on an intracellular response are technically difficult not only because changes of the perfusion medium are required but also because the intracellular response must be held for a long period of time to assure that the entire drug effect is seen and to attempt to reverse the effect by washing the preparation in normal Ringer. In light of these technical difficulties, we generally sought to test the validity of one set of results by pursuing its implications in a different series of experiments rather than attempting to complete an entire dose-response study for any one drug or to obtain a large number of repetitions of any particular experiment. The body of results obtained allowed us to develop a model for synaptic interaction between receptors and ONDs in the dragonfly ocellus consistent with experimental evidence which suggests intriguing possibilities of cellular interactions and provides a basis for future experimental design.

Blocking the Ocellar Nerve Dendrite Response

The light response of the OND was reversibly blocked by 10^{-4} and 10^{-3} g/ml *d*-tubocurarine chloride (curare). The time-course of the response of one cell after perfusion with 10^{-3} g/ml of curare is shown in Fig. 1. The light response was eliminated after 5 min of curare application. In this cell there was no change in resting potential noted during drug application. Shortly after switching to Ringer solution for washing, however, there was a rapid decline in resting potential, indicating loss of the cell.

Fig. 2 shows the response of a different unit before drugs, after a 4-min perfusion with 10^{-3} g/ml curare which blocked the response, and after washing
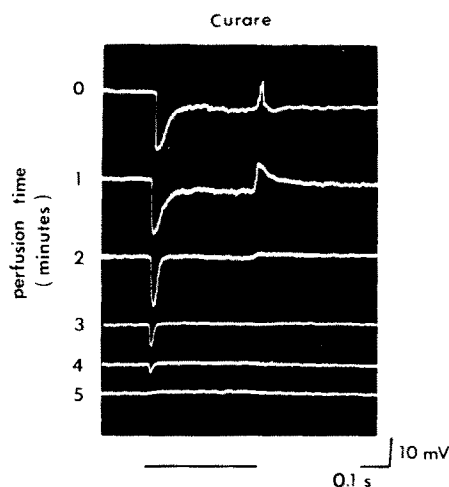

FIGURE 1.   Time-course of curare perfusion. The ocellar nerve dendrite response was progressively decreased until blocked completely by Ringer solution containing 10^{-3} g/ml *d*-tubocurarine (curare) while resting potential remained unchanged. In this and subsequent figures a downward deflection indicates negativity of the active electrode and duration of illumination is indicated by a horizontal bar beneath the responses. Log I = −3.

for 30 min with Ringer solution where a full response was again obtained. Within the first 3 min after curare perfusion was begun, there was a 4 mV positive shift in the resting potential. Perfusion with a lower concentration of curare, 6×10^{-6} g/ml, was begun in five preparations, but no positive results were obtained even after perfusion for 20 min in one of them. In an OND preparation perfused for 25 min with 10^{-4} g/ml atropine sulfate, blocking of the OND response was not observed.

Elimination of the Off Overshoot

The off overshoot was found to be an especially sensitive portion of the OND response. It was altered or eliminated by both picrotoxin and bicuculline. The off overshoot was completely eliminated by 10^{-3} g/ml picrotoxin. The off response was also found to change in a consistent manner before disappearing,

as shown in Fig. 3 in the response recorded 2 min after perfusion was begun where the depolarization after light-off is larger and slower. There is also a fast, negative-going oscillation about 100 ms after light-off, after which there is a slow return toward the resting potential. Washing with Ringer solution for 12 min only restored the off response to this intermediate state.

The off overshoot was also sensitive to lower concentrations of picrotoxin. On two occasions perfusion with 10^{-4} g/ml picrotoxin lasted long enough for
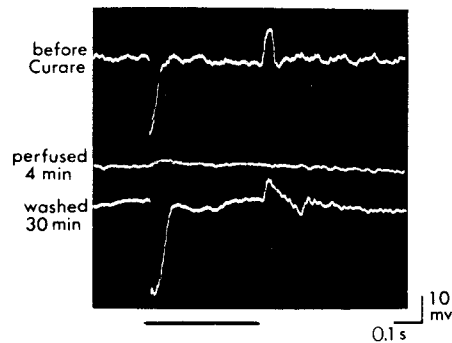

FIGURE 2.   Reversibility of curare effect. The ocellar nerve dendrite response was reversibly blocked by 10^{-3} g/ml curare. Log I = -2.
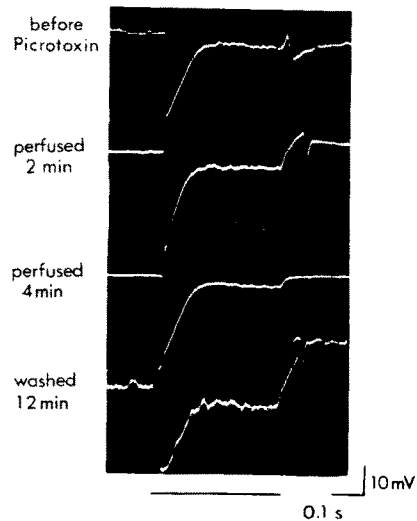

FIGURE 3.   Time-course and limited reversibility of picrotoxin perfusion. The off response of the ocellar nerve dendrite changed in a characteristic way before disappearing when perfused with 10^{-3} g/ml picrotoxin in Ringer. After perfusion for 2 min, the depolarized phase of the off response was increased in size and prolonged; by 4 min, the off response disappeared completely. After 12 min of washing with normal Ringer solution, the off response had not returned to its original form but resembled the intermediate state seen 2 min after the start of perfusion. Log I = -2.

one to see a change in the off response. The change observed was similar to that shown by the off response (Fig. 3) seen after perfusion for 2 min with 10^{-3} g/ml or after washing out 10^{-3} g/ml, and seems to represent a less complete action of the drug.

All 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 perfused with 10^{-5} g/ml picrotoxin, the cell was held long enough to wash with Ringer solution. This concentration of the drug produced the same kind of change in off response as described for 10^{-4} g/ml picrotoxin, but in the 10^{-5} g/ ml case the cell was held long enough to wash with Ringer solution for 40 min and restore the off response to the same phasic form seen before perfusion. With all concentrations of picrotoxin used, the changes became apparent within the first 5 min after beginning perfusion with the drug.

Picrotoxin in the concentrations used did not have a consistent effect on the light-on portion of the OND 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. In these picrotoxin treated units however, the off overshoot in its altered form was a highly reproducible part of the response to successive flashes.

The off response was also eliminated or greatly reduced by bicuculline in concentrations from 3×10^{-5} to 10^{-4} g/ml. Responses before and after 3×10^{-5} g/ml bicuculline perfusion in the most stable preparation obtained are shown in Fig. 4. Changes other than the elimination of the off overshoot are also observed. 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-ms light flash, which will 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 perfusion with bicuculline, in contrast to the apparently random fluctuations in size of the on response which are normally seen. The records on the right in Fig. 4 were obtained after 35 min of perfusion with bicuculline. By this time the responses appeared to have stabilized, and they remained essentially the same for another 15 min, after which the unit was lost before washing was attempted. Changes in the response did not begin to appear until about 15 min after perfusion with bicuculline was begun, and the entire effect shown in the figure required about 30 min. This may be related to the low solubility of the drug in the near-neutral pH of the hemolymph, which may be a significant factor with topical application of the drug. The long perfusion time required to achieve an effect with bicuculline made these experiments especially difficult, and none of the cells were held long enough to study the effects of washing.

Although picrotoxin may not be as specific in its action as bicuculline, they are both known as antagonists of γ-aminobutyric acid (GABA) action. Consequently a study of the effects of GABA perfusion has been undertaken. Results from the best set of records obtained so far are shown in Fig. 5. During a 30-min period of perfusion with 10^{-3} g/ml GABA, there was a gradual increase in

the size of the light response, as well as a change in the form of the off response. After 2 min of washing, the off response regained its oscillatory character. Washing was continued for 6 min, during which there was no change in the on response, and at that point the cell was lost.
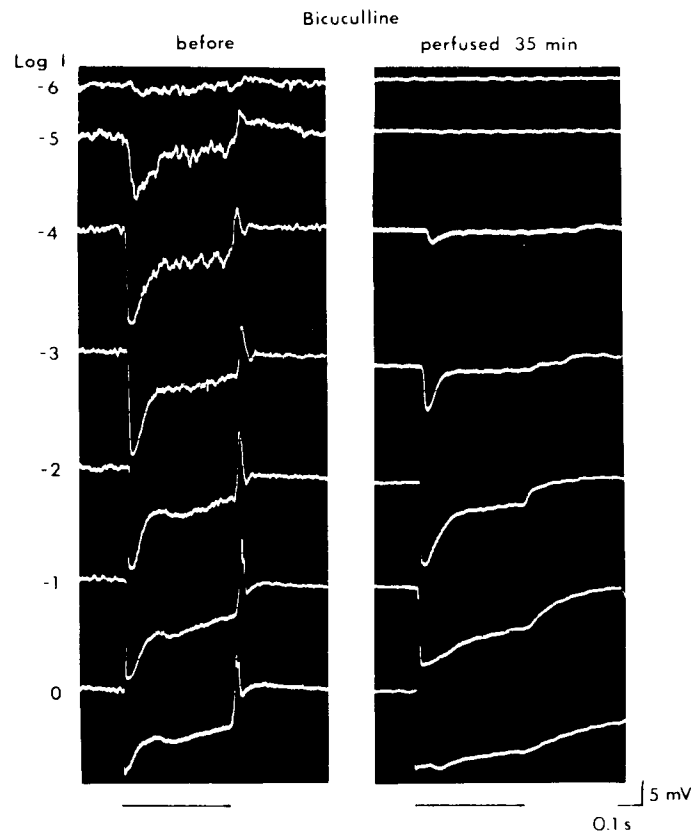

FIGURE 4. Intensity-response series recorded before and after bicuculline perfusion. After perfusion with 3×10^{-5} g/ml bicuculline in Ringer for 35 min, the off overshoot was eliminated, the noise in the dark was reduced, the on transient became smaller, and a flash two log units more intense was required to elicit a threshold response. Log I is indicated to the left of the corresponding pairs of responses.

DISCUSSION

Much of what is known about synaptic transmission has come from the study of synaptic interactions in preparations where transmission is normally evoked by impulses, even though such impulses may be artifically blocked in the course of the experiment. In the dragonfly ocellus, transmission between receptors and ONDs appears to be evoked by slow potentials, inasmuch as the OND response is unaltered by tetrodotoxin, which eliminates impulse activity in the receptors

(Chappell and Dowling, 1972) and in the ocellar nerve (Gallin and Chappell, unpublished observations). Although this type of transmission has not been extensively studied, it appears to be quite significant in vertebrate retinas where intracellular recordings from the first three cell types show no evidence of impulse activity (Werblin and Dowling, 1969; Kaneko, 1970; Murakami and Shigematsu, 1970), and probably in the brain (cf. Schmitt et al., 1976). We discuss, first, evidence bearing on the identity of the neurotransmitters involved,
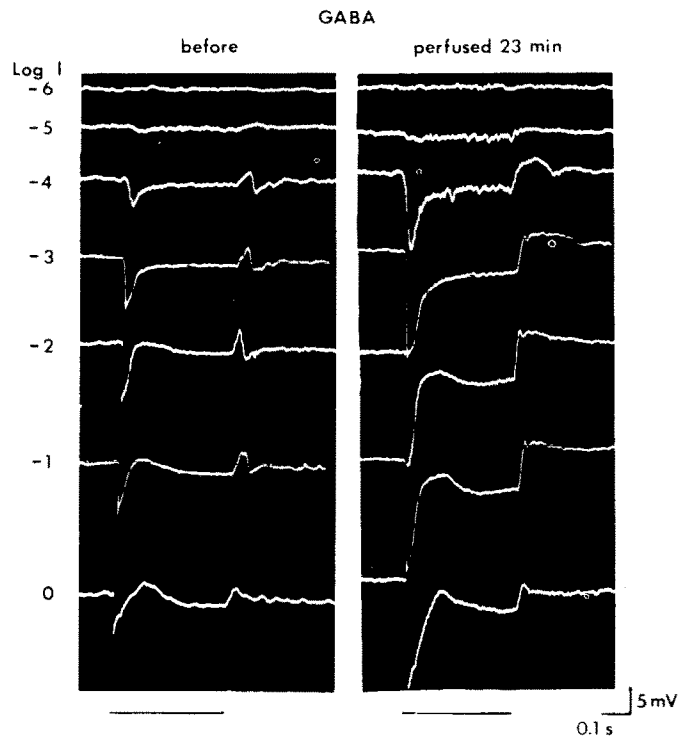

FIGURE 5. Intensity-response series recorded before and after GABA perfusion. After perfusion with 10^{-3} g/ml GABA in Ringer for 23 min, the noise in the dark increased, and the light response became larger. Log I is indicated to the left of the corresponding pairs of responses.

and second, evidence for the existence of at least two pharmacologically distinct types of synaptic events involving receptors and ONDs. We then describe a model for synaptic interaction suggested by our experimental results and discuss mechanisms which may be involved.

Receptor Transmitter

Evidence related to the action and identification of the receptor transmitter has been obtained. Reversal of response polarity across the receptor-OND synapse had already suggested that a chemical transmitter was involved rather than direct electrical coupling (Chappell and Dowling, 1972). The decrease in OND

membrane resistance during illumination reported here supports the hypothesis that the receptor transmitter is an inhibitory transmitter released in increasing amounts by added illumination rather than an excitatory transmitter whose release is reduced by illumination to cause the hyperpolarizing response of the ONDs. This follows from the fact that, in most chemical synapses studied thus far, a decrease in the postsynaptic membrane resistance has been found when either excitatory or inhibitory natural neurotransmitter is present (Coombs et al., 1955; Takeuchi and Takeuchi, 1960; Eccles, 1964; McLennan, 1970a).[1] In addition, release of transmitter at a synapse has always been found to be associated with a depolarization of the presynaptic membrane, and the interpretation of our results hypothesized above is consistent with the idea that the receptor transmitter is also released upon depolarization of receptor terminals.

The OND response to illumination of the receptors can be blocked completely and reversibly (Figs. 1 and 2) by perfusion with curare at a concentration of 10^{-4} g/ml or greater in Ringer solution. This is comparable to concentrations used in other perfused preparations such as the neuromuscular junction where 2×10^{-5} g/ml curare was used to block muscular contraction (Dale et al., 1936) and at the sixth abdominal ganglion of the cockroach where 10^{-4} g/ml curare was found to be the threshold concentration required to antagonize the action of nicotine (Kerkut et al., 1969).

These experiments provide useful information about the ocellus. First, curare may provide a simple means of isolating the receptor response in the ocellus and make it possible to record receptor responses unmodified by feedback from ONDs. In vertebrate retinas, for example, aspartate and glutamate have been useful in isolating receptor responses (Cervetto and MacNichol, 1972; Murakami et al. 1972), although the mechanism is different, in that 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.

Next, curare is widely accepted as a specific cholinergic antagonist, and its blocking action in the ocellus raises the possibility of cholinergic transmission, although curare has been found to antagonize the action of 5-hydroxytryptamine on certain molluscan neurons (Gerschenfeld and Paupardin-Tritsch, 1974). Additional evidence in favor of cholinergic transmission in the ocellus was provided by a study in which assays of ocellar tissue indicated a significant amount of acetylcholine and of choline acetyltransferase activity (Kuhar and Chappell, unpublished observations). Although the identity of the receptor

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[1] This has generally been the assumption made in the interpretation of results of studies on the vertebrate retina (Tomita, 1965; Trifonov, 1968; Toyoda et al., 1969 and 1973) and is supported by some experimental evidence (Dowling and Ripps, 1973; Cervetto and Piccolino, 1974). However, a transmitter which acts by decreasing permeability to certain specific ions and thereby increases membrane resistance seems to remain a logical possibility (Kuffler, 1960; Grundfest, 1961; Kaneko, 1971; Toyoda, 1973). Gerschenfeld and Paupardin-Tritsch (1974), for example, have recently reported that iontophoretic application of 5-hydroxytryptamine to neurons of *Helix* and *Aplysia* causes a depolarization accompanied by a decrease in potassium conductance and a hyperpolarization accompanied by a decrease in sodium and chloride conductance although it is not yet clear whether it is acting directly or by blocking the action of some other transmitter.

transmitter in the ocellus may not be considered completely determined, the results here raise acetylcholine to the status of a most promising candidate for that role, a role which would be consistent with its inhibitory role in other animals (Tauc and Gerschenfeld, 1961; Oomura et al., 1976).

Selective Blocking of a Response Component

Pharmacological agents have been identified which selectively block the oscillatory off overshoot while leaving the basic sustained hyperpolarization essentially intact. This result is consistant with the view that these oscillations are the result of an independent synaptic event involving a different neurotransmitter from the one involved in the sustained hyperpolarization. The off overshoot portion of the OND response was abolished by both picrotoxin and bicuculline (Figs. 3 and 4). Both of these drugs have been shown to antagonize the effects of GABA at synaptic sites.

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 et al., 1970) in the spinal cord (Engberg and Thaller, 1970), in the olfactory bulb (Nicoll, 1971), and in the cerebellum (Woodward et al., 1971). However, inconsistencies in the effectiveness of picrotoxin as a GABA antagonist in the vertebrate central nervous system have also been described (Curtis et al., 1971a). 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 et al., 1970a, 1970b, 1971a, 1971b; Curtis and Felix, 1971; Bisti et al., 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 et al., 1973; Schaeffer et al., 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 et al., 1970; Straughan et al., 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 OND 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 3×10^{-5} to 3×10^{-4} g/ml reduced or prevented the inhibitory action of GABA on the crayfish stretch

receptor, and noted that bicuculline was about 10 times more potent (by weight) than picrotoxin in that preparation.

A Model for Discussion of Synaptic Interaction

Our ability to block selectively the off component of the OND response raised the possibility that these portions of the response could represent contributions from a synaptic event independent from that producing the basic hyperpolarization. One relatively straightforward explanation of the effects of picrotoxin and bicuculline on the OND response would be that the drugs are blocking feedback from the ONDs to the receptors. A more subtle point which evolved from this interpretation is that the overshoot and oscillations at light-off could result from the time lag between cessation of the light stimulus and the return of the transmitters to a dark-adapted equilibrium provided that both transmitters continue to be released in the dark. A similar suggestion was made by Trifonov (1968) to explain the dying oscillations observed in response to trans-retinal current pulses while recording from horizontal cells in a vertebrate retina; however, in that case the feedback onto receptors was thought to be via currents generated by the horizontal cells rather than chemical transmitters released by them.

We have developed a model (Chappell and Klingman, 1974) for the generation of the OND response which involves OND-to-receptor feedback, although we recognize that alternative explanations, such as the possible existence of at least two pharmacologically distinct types of input to the ONDs could be proposed. Our decision to emphasize the role of feedback at this point has been strengthened by preliminary investigations in our laboratory which indicate that the drugs which alter the off response of the OND also effect the off response of the receptor cell. (Stone and Chappell, unpublished observations). Our model suggests that the OND 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 OND is hyperpolarized. Fig. 6 indicates how such a model could account for the typical features of the OND 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 OND. (The number of arrows were arbitrarily chosen to represent amounts of transmitter relative to the dark equilibrium condition.) The receptor transmitter hyperpolarizes the OND and reduces its output of feedback transmitter. The feedback transmitter, on the other hand, increases the receptor terminal's output of its transmitter. The dark-adapted condition represents an equilibrium in the interaction of these two transmitters such that transmitter is released by each cell at a fairly steady rate and a stable "resting" potential is maintained in each cell. The noise which is often recorded in the OND 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 OND. Shortly after light-on (C), the OND, 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 OND potential returns toward the dark level. Eventually an equilibrium or steady state (D) is reached in which the receptor terminal is releasing less transmitter than immediately after light-on (due partly to receptor properties and partly to decreased facilitation by the
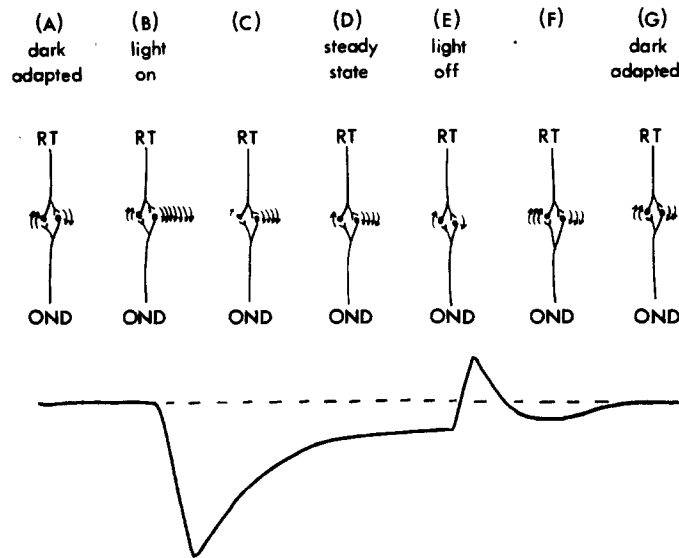

FIGURE 6. Model of feedback interaction. This model was developed on the basis of experimental results to show how a facilitory feedback loop could account for typical features of the ocellar nerve dendrite response. RT and OND represent a receptor terminal and ocellar nerve dendrite, respectively. The 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 potential changes which might be recorded in the ocellar nerve dendrite during this period. (See text for details.)

OND) but more than in the dark, resulting in a small hyperpolarization of the OND. At light-off (E), the receptor potential returns toward the resting level and the output of receptor transmitter is greatly reduced, and consequently the OND 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. Because the OND 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 OND 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 dark potential, as the original equilibrium between the two cells is reestablished (G).[2]

The effect of bicuculline on such a system is presented in Fig. 7. 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 OND in the positive direction. The decrease in noise recorded in the OND in the dark
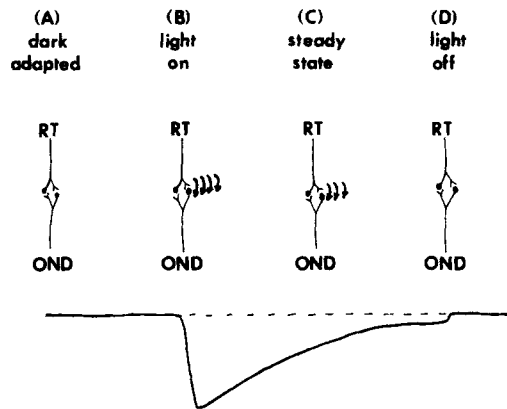

FIGURE 7. Effect of bicuculline on the facilitory feedback loop presented in Fig. 6. Conventions as in Fig. 6. The feedback transmitter is assumed to be blocked by bicuculline. The tracing at the bottom represents the potential changes which might be recorded in a bicuculline treated ocellar nerve dendrite in response to a flash of light. (See text for details.)

after perfusion with bicuculline (Fig. 4) 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 OND hyperpolarization is smaller in magnitude than it is in the normal case. As the light remains on, the transmitter output of the receptor decreases somewhat because of processes in the receptor itself, and the OND 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,

[2] If curare does in fact block the receptor transmitter, it would be expected to shift the resting potential of the ocellar nerve dendrite in a positive direction by blocking the effect of the small amount of receptor transmitter released in the dark. The largest effect on resting potential seen thus far was a 4-mV positive shift which is of the same order of magnitude as normal fluctuations of an untreated cell and is thus inconclusive.

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 OND correspondingly returns to its dark potential without overshoots or oscillations.

In developing the model described above, we found that our results were best explained by postulating a facilitory action of the feedback transmitter although we had expected that the synapse made by the ONDs onto receptor terminals might have an inhibitory function analagous to the lateral and self-inhibition in the *Limulus* lateral eye, especially in light of the role these mechanisms have been shown to play in sharpening the on activity and generating an off overshoot in the eccentric cell response (Ratliff et al., 1963; Hartline, 1969; Ratliff and Mueller, 1957). A model assuming such inhibitory feedback along with a light-released receptor transmitter could, in fact, generate all the features of the normal OND response provided that, as in the model discussed above, both transmitters are released at some equilibrium level in the dark to account for overshoots of the resting potential which can be pharmacologically blocked. If this type of inhibitory feedback occurred in the ocellus, transmission from the receptor terminal to the OND would turn on a process in the latter which would turn down the transmission from the receptor to the OND. This model predicts that blocking the feedback would result in an increase in the size of the OND response, because transmission from the receptor terminal would no longer be turned down. However, as shown in the experiment presented in Fig. 4 in which an OND preparation was perfused with bicuculline for almost 1 h, several stable effects appeared which are inconsistent with such a 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 which would be difficult to explain on the basis of blocking inhibitory feedback. In addition, this inhibitory feedback model requires that the OND release more transmitter as it becomes more hyperpolarized, whereas all other neurons have been found to release transmitter when depolarized. It is on this basis, in fact, that Ozawa et al. (1975) favor the hypothesis that, in the biphasically responding cell (BRC) that they studied in the visual system of the giant barnacle, the hyperpolarizing response recorded results from additional circuitry involving other neurons or feedback connections including the possibility of an interneuron between receptors and the BRC.

In light of these experimental observations and the large body of evidence that release of all known neurotransmitters is increased by depolarization, not hyperpolarization, of a neuron, we favor the facilitory feedback system as a model for synaptic interaction between receptors and ONDs that we have studied in the retina of the dragonfly median ocellus. Although the identity of the feedback transmitter released by the ONDs has not been established, there is considerable documentation of both picrotoxin and bicuculline as GABA

antagonists, and the experiment shown in Fig. 5 is not inconsistent with the possibility of GABA as the transmitter here. Although, at most sites which have been studied, GABA has an inhibitory effect, it has been suggested as an excitatory transmitter as well (Flock and Lam, 1974).

Although the suggestion of direct facilitory feedback between neurons via a natural transmitter is new, evidence suggesting the possibility of presynaptic facilitation of one presynaptic neuron by another has existed for some time (Kandel and Tauc, 1965a, 1965b; Carew et al., 1971; Shimahara and Tauc, 1975; Castellucci and Kandel, 1976). Recent evidence indicates that application of the putative neurotransmitter serotonin results a presynaptic facilitation of transmitter release by some neurons in *Aplysia* (Shimahara and Tauc, 1975; Brunelli et al., 1976). Presynaptic action of a drug at the nerve terminal to facilitate transmitter release is well substantiated pharmacologically in the case of amphetamine. In addition to blocking the re-uptake inactivation of norepinephrine and dopamine, amphetamine can facilitate the release of norepinephrine or dopamine into the synaptic cleft (Stein, 1964; Glowinski and Axelrod, 1965 and 1966; Carlsson et al., 1965; McKenzie and Szerb, 1968).

The Slow Potential and Synaptic Transmission

Any attempt to suggest a possible mechanism for the proposed facilitory feedback at this time would be highly speculative. At the outset, one must make certain assumptions about how synaptic transmission is controlled by slow potential changes. One may be able to learn something of the role of slow potentials alone, however, when presynaptic impulses are prevented experimentally, either by the use of slowly rising currents or tetrodotoxin and the direct effect of the applied polarization may be seen. Under these conditions, transmission, as measured by size of the postsynaptic potentials at the squid giant synapse (Bloedel et al., 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 min, 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. Hence, something as simple as a direct depolarization of the receptor terminal by the feedback transmitter could account for feedback "facilitation" when considered in terms of slow potential interactions.

In summary, the results of the present study indicate that photoreceptors of the dragonfly median ocellus release a transmitter substance upon illumination which has an inhibitory effect on the ONDs, hyperpolarizing them and inhibiting the release of feedback transmitter by the ONDs. There is evidence that the receptor transmitter may be acetylcholine. The feedback transmitter, on the other hand, appears to be facilitory to receptor transmitter release. GABA is a possible candidate for that role.

Being able to modify the OND pharmacologically made it possible to develop

a model for synaptic interaction at this junction which is consistent with a variety of experimental results. Important features of the model include the suggestion that the dark potential of the OND may be determined by a dynamic equilibrium established by synaptic interaction between it and the receptor terminal and the concept of direct facilitory feedback. This feedback could function to enhance sensitivity in the dark (as opposed to reducing sensitivity in the light) and to provide a very sensitive off response or "shadow detector" operable over a wide range of intensities. Both of these characteristics appear to be important features of the dragonfly median ocellar response.

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