ELECTRICAL POTENTIALS FROM THE EYE AND OPTIC NERVE OF *STROMBUS*: EFFECTS OF ELECTRICAL STIMULATION OF THE OPTIC NERVE

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SUMMARY

1. Photic stimulation of the mature eye of *Strombus* can evoke in the optic nerve 'on' activity in numerous small afferent fibres and repetitive 'off' bursts of afferent impulses in a smaller number of larger fibres.

2. Synchronous invasion of the eye by electrically evoked impulses in small optic nerve fibres (apparently the 'on' afferents, antidromically activated) can evoke a burst of impulses in the larger 'off' fibres which propagate away from the eye. Invasion of the eye via one branch of optic nerve can evoke an answering burst in another branch.

3. Such electrically evoked bursts are similar to light-evoked 'off' bursts with respect to their impulse composition, their ability to be inhibited by illumination of the eye, and their susceptibility to MgCl₂ anaesthesia.

4. Invasion of the eye by a train of repetitive electrically evoked impulses in the absence of photic stimulation can give rise to repetitive 'off' bursts as well as concomitant oscillatory potentials in the eye which are similar to those normally evoked by the cessation of a photic stimulus.

5. The electrically evoked 'off' bursts appear to be caused by an excitatory rebound following the cessation of inhibitory synaptic input from photoreceptors which can be antidromically activated by electrical stimulation of the optic nerve.

6. The experimental results suggest that the rhythmic discharge of the 'off' fibres evoked by the cessation of a photic stimulus is mediated by the abrupt decrease of inhibitory synaptic input from the receptors.

INTRODUCTION

The eye of *Strombus luhuanus*, a marine gastropod, is anatomically well developed, visually important to the animal, and yields complex electrophysiological responses to photic stimulation (Gillary, 1974a); it provides an interesting model system for examining the neural processing of visual information. Following amputation of the eye, a new and apparently normal eye is rapidly regenerated in its place (Gillary, 1972). It thus affords the opportunity for following the ontogeny of a relatively complex sense organ with simultaneous physiological and anatomical studies. The work to be described here was directed toward gaining an understanding of the neuronal mechanisms of the mature eye. This information, in addition to being interesting in its own right, is necessary as a basis for examining the development of the regenerating eye.
The extracellularly recorded electrical activity evoked in the eye and optic nerve by photic stimulation has been described previously, along with a brief description of the anatomy of these structures (Gillary, 1974a). Some of these earlier observations, needed for interpreting those to be presented in this paper, will be summarized briefly. Stimulation of the retina evokes an electoretinogram (ERG) which exhibits one or more phasic, cornea-negative 'on' peaks followed by a sustained cornea-negativity of lower amplitude maintained throughout the stimulus. The abrupt termination of illumination, in addition to permitting the cornea-negativity to decline, evokes small regular oscillations in potential (ca. 2–3 Hz at 20 °C) which can persist for more than 20 min in the dark. These oscillations, which are inhibited during photic stimulation, will be referred to as 'repetitive off potentials'. In the optic nerve, photic stimulation also evokes phasic and tonic 'on' activity in a large number of afferent fibres, apparently quite small in diameter, whose discharge pattern reflects the cornea-negativity of the ERG. The abrupt cessation of illumination evokes an 'off' response of repetitive bursts of impulses in a relatively small number of fibres; these 'off' impulses are many times larger in amplitude than the 'on' impulses and appear to arise from fibres which are significantly larger in diameter. Each of the repetitive 'off' bursts coincides with the rapid, initial, cornea-positive phase of each repetitive 'off' potential. Like these potentials, the bursts are inhibited by illumination of the eye. In addition, the larger 'off' fibres often discharge (usually only a single impulse) just after the onset of photic stimulation, during the phasic burst of activity in the small 'on' fibres, although never later, during the sustained 'on' response.

In the studies to be described, electrical stimulation of the optic nerve was employed, as well as the methods of photic stimulation used in the earlier studies. The experimental results indicate that 'off' bursts similar to those evoked by light can be electrically evoked in the absence of photic stimulation by antidromic activation of afferent fibres in the optic nerve. The present results, along with those of the previous paper (Gillary, 1974b), are compatible with the view that the 'off' fibre discharge tends to be rhythmic and associated with oscillatory retinal potentials, and that both types of response are mediated by post-inhibitory rebound following a decrease in inhibitory synaptic input from the receptors. A preliminary report of some of the results has appeared elsewhere (Gillary, 1974b).

METHODS

The experimental procedures for recording from the eye and optic nerve and for delivering photic stimuli were the same as those described previously (Gillary, 1974a). The eye and its attached optic nerve were dissected free from the amputated eyestalk of an adult Strombus luhuanus. During the dissection, the preparation was immersed in 360 mm-magnesium chloride (MgCl2.6H2O), which is isotonic with sea water and causes the muscles of the eyestalk to relax. When isolated, the eye-nerve preparation was placed in sea water, which, unless indicated otherwise, was the bathing medium used during the experiments. Electrical signals from the retina were led to a d.c.-coupled pre-amplifier via a glass-tipped suction electrode applied to the surface of the eye. Suction electrodes with tips of smaller diameter (ca. 50 μm or less) were employed for recording from the optic nerve. The signals were amplified with low-level, differential, a.c. pre-amplifiers, displayed on a cathode ray oscilloscope and photo-
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graphed. Suction electrodes similar to those used for recording activity in the optic nerve were also used to deliver brief electrical stimuli (each usually 0.05 s or less in duration and less than 10 V in amplitude), either singly or in trains.

The types of physiological preparation and the placement of recording and stimulating electrodes are illustrated by the inserts of Figs. 1, 2 and 4. Electrical potentials were recorded from the eye via an electrode applied either to the cornea or to the back of the eye (cf. Fig. 4A). Electrical stimuli were usually delivered relatively close to the cut end of the optic nerve. In certain experiments, a branch of the optic nerve was deliberately cut between the eye and the point of stimulation while leaving the other branches attached to the eye (cf. Fig. 2C). In addition, some experiments were carried out with the isolated optic nerve which had been completely severed from the eye (cf. Fig. 1A).

The preparation was oriented so that flashes of white light from a high intensity, quartz-iodide bulb entered the pupil of the eye. While stimuli with intensities as high as 100 Joule m\(^{-2}\) s\(^{-1}\) could be delivered, intensities several orders of magnitude below this were more usual. Unless stated otherwise, the experiments were conducted with dark adapted preparations at ambient temperatures (20–24 °C) which varied less than several tenths of a degree during a given experiment.

In the experiments concerned with the effects of magnesium chloride on the electrically and photically evoked responses, a stock solution of 360 mM-magnesium chloride (i.e. that used to anaesthetize the animal) was diluted in varying degrees with sea water. Syringes were used to change, within 10 s, the 5 ml of solution which bathed the preparation; several rinses with the new solution were used to ensure complete replacement of the bath.

RESULTS

Impulse activity in the optic nerve

Responses to electrical stimulation. Electrical stimulation of the isolated optic nerve (severed from its eye) can evoke a compound action potential (a.p.) which is propagated along the nerve (Fig. 1A–C). By varying the stimulus intensity the compound a.p. can be seen to be comprised of two components. One is evoked at relatively low stimulus intensities in a population of fibres whose impulses propagate at about 1 m/s (Fig. 1A). These fibres are presumed to have relatively larger diameters and are few in number, since varying the stimulus intensity produces obvious quantal jumps in amplitude. The amplitudes of the impulses which contribute to this component appear identical to those of the large, light-evoked ‘off’ impulses previously described (Gillary, 1974a).

Higher stimulus intensities can excite a second population of fibres, with a mean propagation velocity of approximately 0.3 m/s (Fig. 1B). This slower component, with a higher threshold, is apparently composed of impulses in a relatively large number of fibres with small diameters, since its amplitude appears to vary smoothly with stimulus intensity. The maximal compound a.p. (Fig. 1C) exhibits a slow component which is considerably larger than the rapid component; this further indicates that the fibres with small diameters greatly outnumber those with the large diameters. Since light evokes ‘on’ activity in a relatively large number of small fibres (the propagation velocities of whose small impulses have not yet been determined),
Fig. 1. Effect of stimulation of the optic nerve, either severed from the eye or attached to it. All the records were obtained from the same optic nerve. The preparation is illustrated by the insert in A. The recording site (R) on the nerve was about 4 mm from the point where it entered the eye. Brief stimuli were delivered to the nerve approximately 5.5 mm from the point of entry to the eye (S). Records A–C were obtained after the eye was severed from the optic nerve (at dotted line of insert); records D–F were obtained with the eye still attached to it. Small upward arrows indicate the stimulus artifacts (rapid downward deflexions) in A–C. As indicated by the sizes of the artifacts, the stimulus intensity for A was below that for B, which was below that for C. (Note lower gain in C.) Stimuli of equal intensity were used for traces A and D, B and E, and C and F respectively. Note that the electrically evoked compound action potential exhibits both rapid and slow components (the latter having a higher threshold) and that with the eye attached to the nerve (D–F), an answering burst of impulses (indicated by large arrows in E and F) occurs only in the presence of the slow component of the compound a.p. In this and all subsequent records, an upward deflexion indicates an increase in negativity. The vertical calibration in A also refers to B, D, and E; that in C also refers to F.

these observations are compatible with the view that electrical activation of these 'on' fibres contributes significantly, if not exclusively, to the slow component of the compound a.p.

The records in Fig. 1 D–F illustrate the effects of stimulating near the proximal cut end of the optic nerve while it is still attached to the eye. Stimulation with an intensity capable of evoking only the fast component yielded a response which was essentially identical to that of the optic nerve alone (Fig. 1 D; cf. Fig. 1 A). However, when the threshold of the small, slow fibres was exceeded, a burst of impulses followed the slow component by approximately 20 m/s (Fig. 1 E). The range of amplitudes of the impulses comprising such a burst was identical to that of the light-evoked 'off' impulses described previously. Increasing the size of the slow component (by increasing the stimulus intensity) tended to increase the number of impulses in the burst (cf. Fig. 1 F) and to decrease the latency to its onset.

The direction and velocity of propagation of the impulses in this burst, evoked in response to invasion of the eye by the slow component of the compound a.p., was determined by recording from two locations along the optic nerve. The results are illustrated in Fig. 2 A, in which the upper record was obtained via a recording electrode placed farther from the eye and closer to the stimulating electrode than that used for
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Fig. 2. Simultaneous recording from two points on the optic nerve. All records were obtained from the same preparation under various conditions illustrated by inserts. A, the upper trace was recorded 2.2 mm from the eye and the lower, 1.5 mm from the eye. Stimuli were delivered to the optic nerve about 4.7 mm from the eye. Note that in the lower trace, the three impulses in the answering burst precede those in the upper trace by a fixed latency. B, simultaneous records were obtained from two different branches of optic nerve. The electrodes were 2.5 and 1.5 mm from the eye for the upper and lower traces, respectively. Stimulation was as in A. Note the similarity of the responses. C, the optic nerve was cut so that the compound a.p. invaded the eye via only one branch. Lower trace: record from electrically stimulated branch, which exhibits both a compound a.p. and an 'answering burst' (see text) indicated by the arrow. (Note lower gain.) Upper trace: unstimulated branch contains no compound a.p. (although stimulus artifact displaced base line) but exhibits an answering burst.

While the compound a.p. was propagated towards the eye (as indicated by its longer latency and more diffuse waveform in the lower trace), the impulses in the burst following it were propagated away from the eye, towards the stimulating electrode since the latencies of the three impulses in the lower trace are consistently shorter than those in the upper trace. By filming such responses at higher sweep speeds, their velocity of propagation was determined to be about 1 m/s which corresponds to that obtained from the light-evoked 'off' impulses and the fast component of the compound a.p. Such a burst of large afferent impulses which arises in response to invasion of the eye by a compound a.p. will be referred to as an 'answering burst'.

It appears that the answering burst, presumably composed of impulses in the large 'off' fibres, is evoked by the invasion of the retina by impulses in the slow component of the compound a.p. This component may represent impulses in 'on' fibres stimulated antidromically. By measuring the distance between the recording electrode and the eye, and the latency between the peak of the slow component of the compound a.p. and the burst, and by assuming constant velocities of propagation for the two of 0.3 and 1.0 m/s respectively, it is possible to estimate the delay at the retina between the time of arrival of the slow component of the compound a.p. and the time of burst initiation. While the estimates are necessarily imprecise, they tend to fall in the neighbourhood of 5-15 m/s, depending upon how one chooses the 'peak' of the compound a.p. and the 'time' of the burst. This relatively long delay seems incompatible with the view that the answering burst is triggered primarily by antidromic invasion via the 'off' fibres themselves. While antidromic invasion of a soma can, in certain other systems, cause in the axon a reflected, orthodromic action potential or burst of action potentials (cf. Goldstein & Rall (1974) and Ramon, Joyner & Moore (1975) for theoretical treatments; and Gillary & Kennedy (1969) for a biological example), it seems more likely that in Strombus slower processes are involved in evoking the answering burst, such as chemical synaptic transmission and post-inhibitory rebound (Perkel &
Fig. 3. Repetitive electrical stimuli delivered in the dark and during simultaneous photic stimulation. (Preparation and recording conditions as in Fig. 2A.) Trains of brief electrical stimuli (indicated by small arrows) were delivered to the optic nerve at 20 Hz. In B-D, the stimulus artifacts and compound a.p.s have been deleted in order to help focus attention on the answering bursts. A, responses to the first three stimuli of a 2 s train. B, responses to the last five stimuli of the 2 s train in A. Note the continued discharge. C, the large upward arrow indicates the onset of the 1 s photic stimulus presented during a train which began approximately 1 s before. Note the inhibition of the answering bursts. D, the large downward arrow indicates the end of the photic stimulus in C. Note that the answering bursts return. E, the record begins approximately 1 s after the end of D. The circled small arrows indicate stimuli which evoked substantial answering bursts. Note the periodic modulation in the size of successive bursts. (See text for further details.)

Mulloney, 1974). (It might be noted here that the large impulse seen late in the slow component of the compound a.p. in Fig. 1E, about 15 m/s before the onset of the answering burst, was evoked fairly reliably and may be an example of such a reflected action potential in an 'off' fibre.)

No qualitative differences were seen between records obtained from the various branches of the optic nerve in response to electrical stimulation. This is illustrated in Fig. 2B which shows simultaneous records from two branches. Since no such differences between branches were observed for light-evoked activity either, there is not yet evidence for any functional difference between the branches. If the optic nerve was cut so that the compound a.p. invaded the eye through only certain branches, an answering burst could be produced in a branch which had not propagated a compound a.p. (Fig. 2C), although the response was usually smaller than that of the uncut preparation and required a greater stimulus (and larger compound a.p.). The observation that the pathways for invasion of the eye by the compound a.p. and exit of the answering burst can be different again suggests that initiation of such a burst involves synapses in the retina between different fibres.

Very rarely, a single compound a.p. could evoke two successive answering bursts, rather than the usual single burst. This occurred only in preparations with unusually high excitability (i.e. a very large burst could be evoked by a relatively small compound a.p.). The interval between such bursts was about 0.3 s, which is similar to that between light-evoked 'off' bursts and 'repetitive off potentials' described in the Introduction.

Delivering trains of stimuli to the optic nerve could evoke trains of compound a.p.s and accompanying answering bursts (e.g. Fig. 3A). While the amplitudes of both the
compound a.p. and the answering burst tended to decrease as a function of stimulus frequency and of time after the onset of the train, for appropriate frequencies (e.g. 20 Hz), such accommodation was minimal so that each stimulus in a train lasting several seconds evoked a compound a.p. which was followed by an answering burst. Following the cessation of such a train of stimuli, the optic nerve continued to exhibit a discharge of large impulses with amplitudes identical to those in the answering bursts and the light-evoked 'off' bursts (Fig. 3B). While the post-train discharge usually did not occur in bursts as well defined as those following the cessation of photic stimulation, they did exhibit a rhythmic modulation in impulse frequency; the frequency of this modulation (not visible in the figure) was similar to that characteristic of the light-evoked 'off' bursts (i.e. ca. 2–3 Hz).

Simultaneous electrical and photic stimulation. Illumination of the eye inhibits the generation of answering bursts. Fig. 3C and D illustrate some of the effects of presenting a photic stimulus of constant intensity and 1 s duration during a train of electrically evoked compound a.p.s and their accompanying bursts. Following the onset of illumination, the number of impulses in each burst was reduced (Fig. 3C); after illumination ceased, the number of impulses per burst increased (Fig. 3D). The degree of inhibition correlated best with the cornea-negativity of the ERG rather than with the intensity of illumination, which was always constant throughout a given stimulus. Thus the answering bursts were completely inhibited following the onset of illumination in Fig. 3C, during the large phasic peak of the ERG (not shown), but were only partially inhibited just prior to the cessation of stimulation in Fig. 3D, when the ERG had declined to its steady state value.

The compound a.p.s were not noticeably affected by light; they appear not to be affected significantly by the collision of light-evoked impulses with the invading compound a.p., changes in the refractoriness of the optic nerve under the stimulating electrode following the passage of light-evoked impulses, and the electronic spread of current from the eye along the optic nerve (cf. Gillary, 1970). The results indicate that light exerts its inhibitory effect on the generation of an answering burst not by altering the incoming compound a.p. but rather by a more direct action at or near the site of initiation of the burst.

Following the cessation of a photic stimulus presented during a train of compound a.p.s, the sizes of the answering bursts did not simply return to their steady value prior to the stimulus. Rather, they showed a conspicuous modulation in amplitude. Approximately two cycles of this modulation can be seen in Fig. 3E. For two clusters of electrical stimuli (indicated by circled arrows), each stimulus is followed by a strong discharge of large impulses, while the stimuli surrounding these clusters (indicated by uncircled arrows) are followed by weaker discharges or none at all. The frequency of this modulation (about 3 Hz) is similar to that of the repetitive 'off' potentials, described earlier, and their concomitant light-evoked 'off' bursts. As can be seen from the record, the impulses still tended to be organized as answering bursts, although the longer duration of some of the bursts caused some impulses to occur after the following stimulus artifact and during the next compound a.p.

In spite of the intermittent periods of silence seen in the record of Fig. 3E, the frequency of the large impulses averaged over a second or more was significantly greater than such an average value preceding the photic stimulus. It was also greater
Fig. 4. Potentials from the eye during optic nerve stimulation. A, responses from the dark-adapted preparation (illustrated in insert). The optic nerve was stimulated 5-5 mm from the eye. Upper trace: optic nerve response to a single electrical stimulus, recorded 3 mm from the eye, showing stimulus artifact, compound a.p. and answering burst. Lower trace: simultaneous potential recorded from the back of the eye. Note the deflexion in potential (ca. 0.04 mV) which corresponds with the answering burst in the upper trace. B, the same preparation and recording conditions as in A during elicitation of a train of compound a.p.s at 30 Hz, whose onset and cessation are indicated by the upward and downward arrows, respectively. Note the small repetitive potentials following the train. The simultaneous record from the optic nerve, not included in the figure, exhibited bursts of 'off' fibre impulses which were synchronous with the small repetitive potentials. C and D, light adapted preparation, different from above, exhibiting ongoing repetitive 'off' potentials (see text). Arrows indicate, as in B, trains of compound a.p.s evoked in the optic nerve at 20 Hz. The three traces in C are continuous. Note the decreased amplitude of the repetitive 'off' potentials during the train and, in C, the altered post-train pattern of oscillation. The time calibration for B and C are the same; vertical calibration for the lower trace of A also applies to B–D. (See text for further details.)

than the average frequency at which light-evoked 'off' impulses would have been elicited in the absence of a train of compound a.p.s. Thus, with regard to the initiation of the large impulses, there appears to be a summation of the effects of invasion of the eye by a compound a.p. and the release from photic inhibition. The above results suggest that the generation of light-evoked 'off' impulses and the initiation of the impulses in the answering burst involve a common process.

Potentials from the eye in response to optic nerve stimulation. If the answering bursts and light-evoked 'off' bursts are indeed generated by a common retinal process, one might expect small potentials from the retina to accompany answering bursts, just as the repetitive 'off' potentials accompany light-evoked 'off' bursts. Fig. 4A and B show examples of such potentials which were recorded from the back of the eye rather than from the cornea; this was also the most effective way of recording repetitive 'off' potentials. The initiation of a single answering burst (Fig. 4A) evoked a simultaneous deflexion in potential at the eye which was similar to one of the repetitive 'off' potentials in terms of its amplitude (ca. 0.04 mV) and its initial negativity at the back of the eye. Evoking a train of answering bursts (Fig. 4B) caused a somewhat erratic but sustained negative deflexion in potential at the back of the eye during the train. Following cessation of such a train, repetitive oscillations in potential often occurred which were similar to the light-evoked repetitive 'off' potentials in terms of their
amplitude, waveform and frequency. The simultaneous activity in the optic nerve (not shown in the figure) exhibited answering bursts during the train and, following the train, bursts of large impulses which were synchronous with each negative deflexion in potential at the back of the eye. (Similar optic nerve activity is illustrated in Fig. 3A and B.) Thus, electrical stimulation is capable of evoking from a quiescent dark-adapted eye, electrical potentials which are very similar to the repetitive ‘off’ potentials evoked by photic stimuli.

As described earlier, following photic stimulation (which light-adapts the eye), repetitive ‘off’ potentials can persist in the dark for more than 20 min. Evoking a train of answering bursts in such a light-adapted preparation can affect the ongoing pattern of the repetitive ‘off’ potentials. In Fig. 4C, the train of electrical stimuli caused a marked decrease in the amplitude of these potentials during the train although the frequency remained relatively unchanged. Following the train, the potentials exhibited rhythmic amplitude modulation with a periodicity of about 0.2 Hz. In Fig. 5D, the amplitude of the repetitive ‘off’ potentials was also decreased during the train of electrical stimulation, although the amplitude modulation following the train was not as dramatic as in Fig. 4C, possibly because the trains were shorter in duration. Invasion of the eye by a train of compound a.p.s did not have a noticeable effect on the cornea-negative phase of the ERG, as it does in Helix (Gillary, 1970).

The experiments illustrated in Fig. 4 show that, like the light-evoked ‘off’ bursts, the answering bursts, evoked by compound a.p.s which invade the retina, are associated with small, transient increases in negativity at the back of the eye. They also show that the oscillation pattern of ongoing repetitive ‘off’ potentials in a light-adapted eye can be influenced by the elicitation of answering bursts. These results, along with those presented previously, support the view that the initiation of answering bursts and light-evoked ‘off’ bursts involve a common process which is reflected by the small retinal potentials.

**Effects of magnesium chloride**

Magnesium ions have been shown to inhibit chemical transmission between excitable cells (e.g. neuromuscular transmission; cf. del Castillo & Engbaek, 1954). In order to help assess the role of chemical synapses in producing the electrical responses described above, the susceptibility of these responses to inhibition by magnesium chloride was examined. In these experiments, the sea water which normally bathed the preparation was replaced with solutions containing proportions of isotonic magnesium chloride in sea water (see Methods) while noting the responses to periodic photic and electrical stimuli.

The various responses evoked by these stimuli showed markedly different susceptibilities to magnesium chloride. The light-evoked, cornea-negative phase of the ERG and the electrically evoked compound a.p. were quite insensitive to such treatment and could, for example, persist for more than an hour in the presence of 50% magnesium chloride in sea water (approximately 200 mM-Mg²⁺, or about four times the Mg²⁺ concentration in sea water). Light-evoked ‘on’ activity in the optic nerve was also relatively insensitive to treatment with magnesium chloride. On the other hand, the light-evoked repetitive ‘off’ potentials and their accompanying ‘off’ bursts as well as the answering bursts elicited by invasion of the eye by compound a.p.s, were very
sensitive to magnesium chloride anaesthesia; these could be abolished within several minutes after immersion in 50% magnesium chloride in sea water. This inhibition was fully reversible; the responses returned rapidly (within minutes) upon reimmersion in pure sea water. These results are compatible with the view that impulse activity in the large ‘off’ fibres is mediated by activity at chemically transmitting synapses.

**Retinal movement**

In addition to evoking electrical activity in the retina and optic nerve, photic stimulation of the eye can elicit slight but distinct retinal movement which occurs with a time course similar to that of the ‘on’ response of the eye and optic nerve and appears to be mediated by the contraction of small muscle fibres associated with the capsule of the eye. This movement is most easily observed in the enucleated eye or isolated retina and is therefore not dependent on central efferent control but, rather, appears to be mediated more directly by photically excited retinal neurones. The retinal movement can be reversibly inhibited by magnesium chloride, as can the light-evoked ‘off’ bursts and electrically evoked answering bursts in the large fibres in the optic nerve. However, since the movement and the light-evoked ‘off’ bursts have radically different temporal characteristics and since the answering bursts, both singly and in trains, can be evoked by stimulation which does not cause visible retinal movement, it is very unlikely that the ‘off’ fibres are mechanoreceptors whose discharge depends on this retinal movement. Rather, this movement appears to be an independent by-product of photoreceptor excitation.

**DISCUSSION**

The results of an earlier paper (Gillary, 1974a), in which only photic stimulation was employed, indicated that in the retina and optic nerve of *Strombus*, there were two types of neurone with light-dependent activity; these mediated ‘on’ and ‘off’ responses, respectively. Activity in the ‘on’ fibres of the optic nerve was associated with corneal-negativity of the ERG; these fibres, with relatively small diameters, appeared to be processes of the photoreceptors. The ‘off’ fibres, larger in diameter and fewer in number, responded to the cessation of photic stimulation by discharging with repetitive bursts of impulses which were associated with rhythmic oscillations in potential recorded from the eye. Since the ‘off’ discharge and the concomitant oscillations in potential (termed repetitive ‘off’ potentials) were inhibited during the light-evoked, cornea-negative phase of the ERG, it was surmised that the ‘off’ activity reflected a release from light-evoked inhibition, which on the basis of certain anatomical features of the retina (also described in that paper), was believed to be synaptically mediated rather than primary.

The results of the present studies, which employed electrical stimulation of the optic nerve as well as photic stimulation of the eye, corroborate and refine the picture of the retina which emerged from the earlier studies. They show that bursts of orthodromic impulses in the ‘off’ cells can be evoked in the absence of photic stimulation by electrical stimulation of the optic nerve when it exceeds the threshold of the fibres with smaller diameters. The electrically evoked bursts in the ‘off’ fibres (termed answering bursts) are similar to those initiated by the cessation of photic stimulation with respect to their impulse composition and their ability to be inhibited by illumination of the eye.
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The answering bursts are also similar to the light-evoked 'off' bursts in that both are associated with small rhythmic retinal potentials in the eye. The ability of the electrically evoked response of the 'off' fibres to summate with that evoked by the cessation of a photic stimulus further indicates that both types of response share a common origin. Such a common origin is also suggested by the similarity in susceptibility of both electrically and photically evoked 'off' fibre activity, as well as their accompanying retinal potentials, to inhibition by magnesium chloride.

The initiation of impulses in the 'off' fibres appears to be mediated by synaptic input from a different population of neurones. This is supported by the observation that an answering burst in the large fibres is apparently elicited by the invasion of the retina by electrically evoked impulses in fibres with much smaller diameters. That the answering burst can be evoked in one branch of optic nerve by invasion via another branch also argues that the responding neurones are different from those stimulated. The relatively long latency between the time of retinal invasion and the initiation of an answering burst, along with the results of the experiments involving magnesium ion inhibition, are compatible with the view that chemically transmitting synapses are involved in the initiation of 'off' impulses. For the present, it seems permissible to presume that the neurones which provide the synaptic input which gives rise to an answering burst in the 'off' cells are 'on' afferents antidromically activated, rather than efferent fibres. While efferent fibres to the retina appear to occur in other molluscan species (Young, 1962; Boycott et al. 1965), there is as yet no concrete evidence that they occur in Strombus, and they are not needed to explain the current results.

The studies reported here also indicate that the electrical activity associated with the 'off' cells tends to be rhythmic under a variety of conditions. The 'off' fibre discharge following repetitive invasion of the eye by electrically evoked impulses exhibits a modulation in frequency similar to that of the light-evoked 'off' bursts (ca. 2–3 Hz at 20 °C). The small rhythmic potentials recorded from the whole eye following photic or electrical stimulation show a similar periodicity. In addition, during repetitive invasion of the eye by apparently uniform compound a.p.s, the cessation of a photic stimulus can cause a periodic modulation of the number of impulses in each of the repetitively evoked answering bursts; this modulation, whose frequency is also 2–3 Hz, suggests a rhythmic variation in the excitability of the 'off' cells. This tendency of the 'off' cells to discharge with repetitive bursts at a 'natural' frequency which is characteristic of the repetitive 'off' potentials may reflect some measure of endogenous rhythmicity, which many other types of neurones exhibit (for examples, see Gillary & Kennedy, 1969; Kater & Kaneko, 1972; Barker & Gainer, 1975).

The experimental results described above indicate that at least two types of neurone, the receptors and 'off' cells, occur in the retina and optic nerve of Strombus. The receptors, which possess distal segments composed of microvilli (Gillary, 1974a) appear to be similar to other molluscan photoreceptors which, in response to photic stimulation, exhibit a graded depolarization and concomitant afferent impulses in optic nerve fibres (Jacket, 1969; McReynolds & Gorman, 1970; Alkon & Fuortes, 1972; Mpitsos, 1973). In some if not most gastropods, these afferent fibres appear to be direct processes of the receptors themselves, rather than the axons of second order retinal neurones (Bullock, 1965; Eakin & Brandenburger, 1967; Stensaas, Stensaas & Trujillo-Cenoz, 1969). The 'off' cells, which are apparently fewer in number than
the receptors, also have axons which run in the optic nerve. These 'off' cells may bear some homology to a type of neurone, not a photoreceptor, which has been postulated on anatomical grounds to occur in the retina of certain gastropods (cf. Kataoka, 1975; Brandenburger, 1975).

While the experimental results are by no means conclusive, they are compatible with the view that the 'off' cells receive inhibitory synaptic input from the receptors. This view is in agreement with the results of recent experiments on the retina of *Strombus* in which intracellular microelectrodes were employed (Quandt & Gillary, 1975). These studies have revealed two classes of neurone, those which are depolarized by photic stimulation and appear to be the photoreceptors, and a less frequently encountered type of neurone which is hyperpolarized by photic stimulation and appears to correspond to the 'off' cell described in this paper. (The intracellular studies will be described more fully in a later publication). Light-induced depolarization of photoreceptors apparently can give rise to postsynaptic hyperpolarization and inhibition in the retina of another gastropod, *Hermisenda* (Alkon & Fuortes, 1972).

The discharge of impulses in the 'off' cells following the cessation of photic stimulation appears to arise from post-inhibitory rebound, which has been shown to effect excitation in a variety of different neurones (cf. Perkel & Mulloney, 1974). The electrically evoked answering burst could also arise from post-inhibitory rebound, for example following brief synchronous activation (via antidromic invasion) of inhibitory synapses of receptors on to the 'off' cells. The relatively slow time course of the post-inhibitory rebound, along with a synaptic delay, could account for the relatively long latency between antidromic retinal invasion and the initiation of an answering burst. Post-inhibitory rebound may also serve to initiate and sustain the rhythmic 'off' bursts and retinal potentials recorded in the dark following invasion of the retina by a train of electrically-evoked compound a.p.s (cf. Figs. 3B and 4B).

As mentioned in the Introduction, the 'off' fibres often discharge a single impulse immediately after the onset of photic stimulation, although never later, during the sustained 'on' activity. This discharge does not necessarily imply that light can stimulate the 'off' cells directly. For example, the discharge may be due to relatively weak excitatory input from the receptors, mediated by electronic coupling, which is quickly overridden by inhibitory synaptic input. The 'off' cells may also be excitatorily coupled to each other. This would explain their apparently synchronous activity, as reflected in the repetitive 'off' bursts and the concomitant oscillatory potentials which can be recorded from the eye with a large extracellular electrode.

Many observations regarding the retina of *Strombus* await explanation. For example, the presence of two or more phasic 'on' peaks which can be exhibited by the ERG and intracellular potentials (Gillary, 1974a; Quandt & Gillary, 1975) requires additional study. The possibility that more than one type of photoreceptor is present in the retina of *Strombus*, as appears to be so in other gastropod retinas (Hughes, 1970; Alkon & Fuortes, 1972; Brandenburger, 1975), also needs to be explored further. Continuing efforts, which include intracellular recording from single retinal neurones, intracellular staining techniques, and electron microscopy, are being made to further our understanding of the retina of *Strombus*.
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