

ELECTRICAL ACTIVITY AND STRUCTURE OF RETINAL CELLS OF THE *APLYSIA* EYE: I. SECONDARY NEURONES

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SUMMARY

1. Intracellular recordings were made from secondary neurones and photoreceptors of the *Aplysia* eye concurrently with extracellular recordings from the optic nerve. These cells were injected with Lucifer yellow to reveal their structure after they were typed according to electrical activity. Secondary neurones are described in this paper.

2. All secondary neurones injected with Lucifer yellow were in the outer, non-receptor layer of the retina. Each had an axon in the optic nerve, short dendritic processes on the soma, but no distinct photoreceptive apparatus. Dye coupling between secondary neurones and between secondary neurones and photoreceptors was observed.

3. Secondary neurones had pacemaker potentials and action potentials (APs) correlated 1:1 with the optic nerve compound action potentials (CAPs) during spontaneous dark and light evoked activity. It is deduced that the secondary neurones are the output neurones of the circadian clock system of the eye.

4. Secondary neurones appear to be electrically coupled to each other and to some photoreceptors, since blocking chemical synapses with high Mg^{2+} saline did not block the spontaneous or light evoked activities, and antidromic activation of the secondary neurones produced a compound input dependent in amplitude on stimulus voltage.

5. Backfilling the optic nerve with cobalt revealed filled secondary neurones, 2 photoreceptor types and a small non-receptor cell type suggesting that most of these retinal cells have axons in the optic nerve.

INTRODUCTION

Each eye of the marine gastropod *Aplysia* exhibits a circadian rhythm of neural activity in isolation (Jacklet, 1969*a*) or in the intact animal (Block, 1979) and has the photoreceptors for resetting the phase of the circadian rhythm (Eskin, 1971; Jacklet, 1974; Benson & Jacklet, 1977). It also responds to light pulses with the transient activity (Jacklet, 1969*b*) of a simple photoreceptor. In this paper and the following one (Jacklet & Rolerson, 1982), the retinal cells responsible for these functions are investigated.

The eyes of *Aplysia* are situated just anterior to the rhinophores on the head of the animal and are rather small and poorly developed compared to the eyes of another marine gastropod, *Strombus*, which exhibits complex visually-guided behaviour (Gillary & Gillary, 1979). The visually-guided behaviour of *Aplysia* is not obvious. However, the eyes are extremely important in maintaining the circadian rhythm of locomotion exhibited by the animal, although some eyeless animals have weak rhythms (Lickey & Wozniak, 1979; Strumwasser *et al.* 1979). The control of locomotion is mediated by neural connexions from the eye to the brain (Lickey *et al.*, 1976) although peptides are released rhythmically from the eye (Strumwasser *et al.* 1979).

Previous studies of the eye have shown that it is moderately complex. It contains about 5000 photoreceptors and neurones (Jacklet, 1973*a*) and has about the same number of fibres in the optic nerve. Several morphological types of photoreceptors and a secondary neurone type are known (Jacklet, 1969*b*; Jacklet, Alvarez & Bernstein, 1972; Luborsky-Moore & Jacklet, 1977). The most conspicuous photoreceptor type is filled with densely-packed, clear, 500 Å vesicles and has a prominent microvillous distal photoreceptive segment. These photoreceptors are stained with Procion yellow dye when the optic nerve is backfilled (Jacklet, 1976). Intracellular recordings combined with dye injection have revealed depolarizing receptors in the innermost retinal layer (Jacklet, 1976). They probably correspond to the microvillous receptors. Other recordings showed neurones that spiked in synchrony with the compound action potentials (CAPs) in the optic nerve. They were in the outermost layer of the retina and corresponded to the clustered secondary neurones stained by backfill. They have been designated 'D' neurones (Jacklet, 1976) since they are spontaneously active in darkness and contribute to the CAP activity, which changes its frequency as directed by the circadian clock (Jacklet, 1981). They have dense-core, 1000 Å vesicles in the cytoplasm and are coupled by gap junctions (Luborsky-Moore & Jacklet, 1977).

More recent studies (Strumwasser *et al.* 1979) indicate that there are several additional cell types in the retina, called upper retinal cells and lower retinal cells. In *Aplysia punctata* an additional ciliated photoreceptor is known (Hughes, 1970). This photoreceptor type with occasional 9+2 cilia and tufts of short microvilli on the distal segment has been seen also in *Aplysia californica* (Jacklet & Colquhoun, unpublished observation). It may be the upper retinal cell described by Strumwasser *et al.* In this paper the secondary or 'D' neurones are considered. The photoreceptors are described in a companion paper (Jacklet & Rolerson, 1982). Preliminary abstracts of the results of the present study have appeared (Jacklet, 1979, 1980*a*).

METHODS

Eyes with attached optic nerve were dissected from *Aplysia californica* weighing 100–300 g. obtained from Pacific Bio-Marine of Venice, Ca. Eyes were treated with protease (Sigma, bacterial type VII) for 10 min (1 mg/ml) to soften the connective tissue sheath and to stop photically-induced contractions of muscle cells in the sheath. This treatment caused no obvious damage to photoreceptors or neural elements. Eyes were placed in a chamber (7 ml) containing artificial sea water (ASW). The optic

nerve was pulled into a tubing electrode for whole nerve recording and the eye was pinned to allow intracellular recording.

Micropipettes filled with 2.5M-KCl having tip resistances of 50–100 M Ω were used or similar electrodes filled with 3% Lucifer yellow CH (5x increased resistance). Lucifer yellow was a gift from Walter Stewart and was used as described by him (Stewart, 1978). Electrodes were coupled to a WPI 701 electrometer equipped with a Breakaway Box for passing current (5–10 nA hyperpolarizing pulses of 500 ms at 1 Hz) to inject dye. Recordings from a single cell and the optic nerve were displayed on a Tektronix 5113 storage oscilloscope and a Grass polygraph. Electrodes were guided with a Leitz micromanipulator. After a good impalement was made, the recording was examined for spontaneous dark activity and then tested with a light pulse. White light from a 6 V tungsten source was used, led to the eye with a fibre optic light guide and controlled by an electromechanical shutter. A standard intensity was 1 mW/cm², at the tip of the light guide. Eyes were studied by applied current through the electrode, electrical stimulation of the optic nerve, and changing the ASW to a high Mg²⁺ saline (0.1 mM-Ca²⁺ – 128 mM-Mg²⁺) to test for chemical synapses, if the recording lasted long enough. Cells studied with Lucifer yellow electrodes were injected with the dye immediately after obtaining a stable recording and determining the response type. They were injected for 10–15 min then fixed within 15 min with formalin ASW (1:7), for 1 h, dehydrated quickly in ethanol, cleared in xylene and embedded in paraffin. They were sectioned (usually 8 μ m), mounted using a low fluorescence mountant (Harleco or Entellan) and viewed with an American Optical microscope equipped with a model 2070 Vertical Fluorescence unit using a BG12 exciter filter and a OG515 barrier filter. Sections were photographed immediately with Ectachrome ASA 400 film because of rapid fading of the fluorescence, particularly in the fine processes. The morphology of the injected cells was reconstructed from Ectachrome slides of the serial sectioned tissue.

The optic nerve was backfilled with cobalt by placing the cut end in a pool of 1 M cobaltous acetate. The cobalt was separated from the rest of the eye, in ASW, by a vaseline seal. Eyes were backfilled for 12–24 h, rinsed, treated with ammonium sulphide for 3 min, rinsed, fixed in Carnoy's, dehydrated and embedded in paraffin, sectioned, and intensified according to a modified Timm's procedure (Bacon & Altman, 1977).

RESULTS

The morphology of the eye is shown in Fig. 1. It is 0.7 mm in dia. and has a central lens surrounded by a pigmented, layered retina (R). The lens nearly fills the cup of the eye leaving a thin strip of vitreous body between the lens and the retina. The retina consists of a pigmented inner layer containing the distal segments of photoreceptors and pigmented support cells (Jacklet *et al.* 1972), a nuclear layer containing the nuclei of those cells, and an outer layer containing fibres from the photoreceptors as well as clusters of secondary cells (Luborsky-Moore & Jacklet, 1977). The optic nerve extends to the cerebral ganglion and contains afferent fibres from the photoreceptors and secondary cells, as well as efferent fibres from the cerebral ganglion (Eskin, 1971).

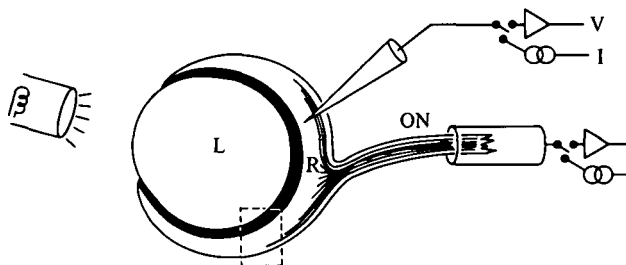


Fig. 1. Diagram of the eye, and the stimulating and recording arrangements. The eye is 0.7 mm in dia., has a large central lens (L) and a complex layered retina (R), including a dark pigmented layer. A tubing electrode on the optic nerve (ON) is used for electrical stimulation or recording. Intracellular electrodes are used to record from a single cell (V) and stimulate (I), or inject Lucifer yellow dye by iontophoresis. Fibre optics and a tungsten lamp are used for photic stimulation. Dotted rectangle shows position of Fig. 2 in the retina.

Backfilling the optic nerve with cobalt

Most attempts to backfill the retinal cells by the movement of cobalt up the axons of the optic nerve were unsuccessful or produced only weak staining. Some of the strongly stained cells are shown in Fig. 2. A large photoreceptor cell is darkly stained in Fig. 2A. Its distal segments extend through the pigmented layer toward the lens, ending in the vitreous body. Several proximal processes extend from the soma and one approaches a large secondary neurone (arrow in the outer layer of the retina). Several other distal segments, and faintly stained nuclei are visible in Fig. 2A. In Fig. 2B an adjacent part of the retina is shown. It has the same layering as the other section and shows a pair of photoreceptors as well as three secondary neurones (arrows). The photoreceptors may be different in type from the one in Fig. 2A, having only one conspicuous process extending from the soma. These results generally confirm the results obtained by backfilling with Procion Yellow (Jacklet, 1976), namely, secondary neurones as well as large multipolar photoreceptors are stained readily. Other types of cells are also filled including the smaller photoreceptor type shown in Fig. 2B and other smaller ($< 5 \mu\text{m}$) neurones in the nuclear layer and along the fibre tracts (not shown).

Intracellular staining of secondary neurones with Lucifer yellow dye

A secondary (or 'D') neurone, unlike any other cell, fires action potentials (APs) in unison with the CAPs recorded from the optic nerve, both in darkness and evoked by a light pulse as shown in Fig. 3. The secondary neurones are therefore the output neurones of the circadian rhythm. A shallow pacemaker depolarization is typically observed between dark spontaneous APs in a 'D' neurone (Fig. 3).

Secondary neurones that were successfully filled with Lucifer yellow did not have a distal photoreceptive process, the soma was in the outer-most layer of the retina, short processes extended from the soma and each neurone had a major axon that entered the optic nerve. Reconstructions from serial sections showed a shape generally like that in Fig. 5A. Branching of the axon was sometimes observed (Fig. 5C). Cells showed typical secondary neurone activity (Fig. 5C). Dye coupling to other secondary neurones and photoreceptors was observed (Fig. 4, Fig. 5B).

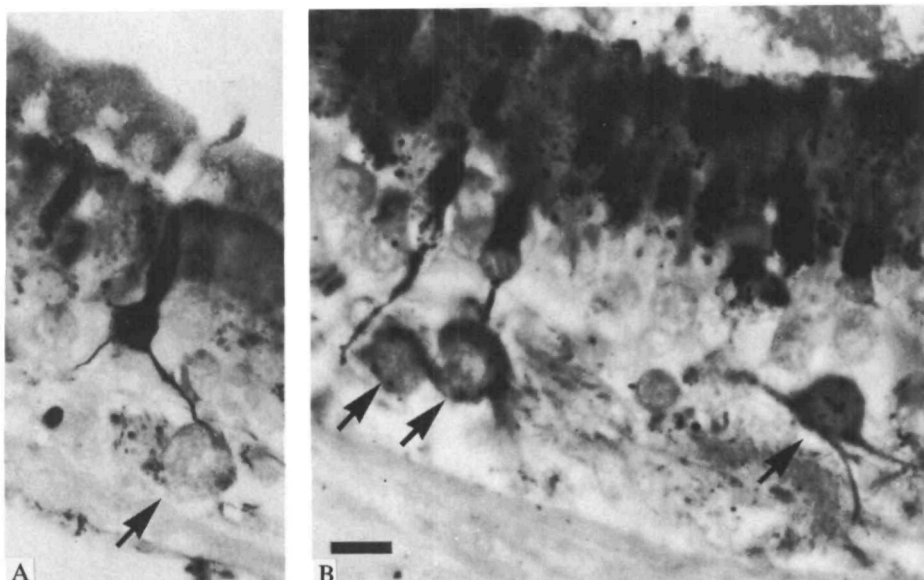


Fig. 2. Retinal cells stained with cobalt by backfilling the optic nerve. Upward is toward the lens. In (A) a multipolar receptor cells is shown with its distal segment extending through the pigmented layer to the vitreous body adjacent to the lens. One process of the receptor cell extends to a faintly stained secondary neurone (arrow). Two receptors stained in adjacent retina are shown in (B). The single apical process from each receptor extends toward a pair of secondary neurones (arrows). Another secondary neurone (arrow) with several processes is shown to the right. Other stained cell bodies are visible as well as patches of neuropile. Sections are $8\text{ }\mu\text{m}$ thick. Scale for (A, B) is $10\text{ }\mu\text{m}$.

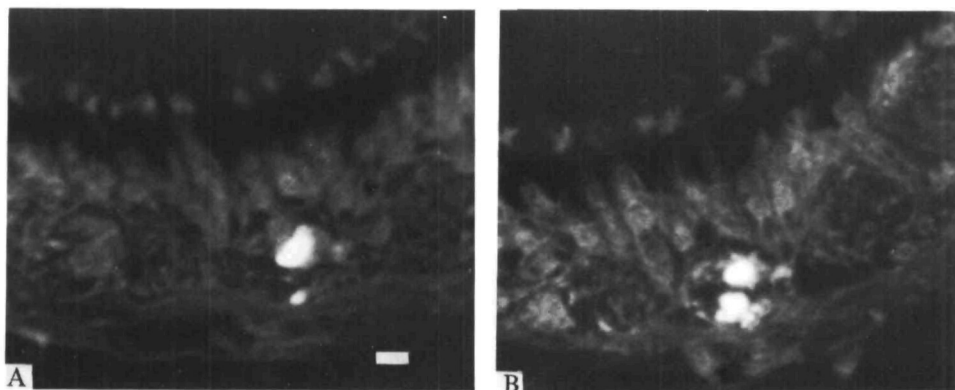


Fig. 4. Lucifer yellow filled neurones of the retina. The orientation of the retina is similar to Fig. 2. The dark pigmented layer is faintly visible. The soma of one secondary neurone and another bright spot below it is shown against the retinal background autofluorescence. The adjacent serial section ($8\text{ }\mu\text{m}$) in (B) shows that the lower spot in (A) is another filled secondary neurone. The complete reconstruction is shown in Fig. 5B. Scale for (A, B) is $10\text{ }\mu\text{m}$.

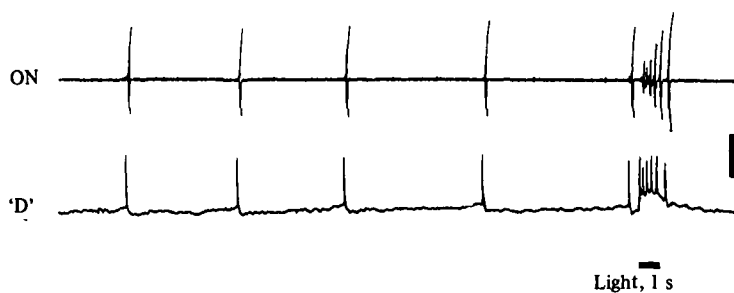


Fig. 3. Characteristic activity of a secondary ('D') neurone. The intracellularly recorded (lower trace) shallow pacemaker potentials and APs are correlated 1:1 with the spontaneous dark CAPs (upper trace). Action potentials are correlated with light evoked CAPs, whose sizes are graded in amplitude. Scales: CAPs, $45 \mu V$; spikes, 10 mV; time 1 s, polygraph recording.

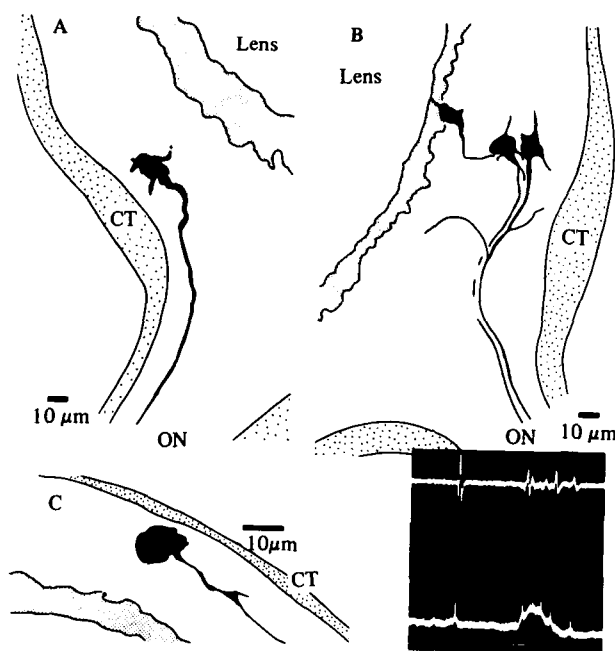


Fig. 5. Lucifer yellow injected secondary neurones, reconstructed from serial sectioned tissue. A typical monopolar secondary neurone with an axon in the optic nerve and many short processes extending from the soma is shown in (A). Two secondary neurones and a photoreceptor that filled even though only one secondary neurone was injected is shown in (B). Another secondary neurone less completely filled than the others, and the recordings from it showing the characteristic activity, APs (lower trace) correlated 1:1 with dark CAPs and light-evoked CAPs are shown in 5C. Resting potential is 35 mV, spikes 15 mV, light 1 s. The pigmented layer is finely stippled and connective tissue (CT) is coarsely stippled.

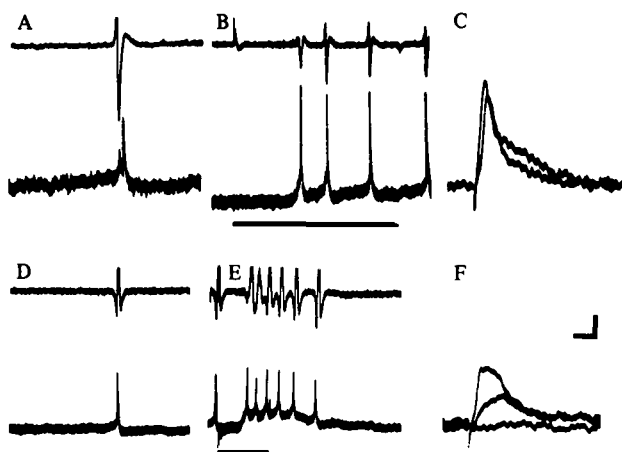


Fig. 6. Secondary neuron and optic nerve activities. Spontaneous dark (A), light-evoked (B) and optic nerve stimulated activity (C) of one neurone (resting potential 35 mV) is shown and similar activity of another (D–F). CAPs (upper traces) are neg. down (A, B) and neg. up (D, E). Optic nerve stimulation at 15 V and 50 V, 0.5 ms in (C); 25 V, 30 V, 35 V, 0.5 ms in (F). Blip on optic nerve trace in (B) at light-on is artifact. Scales: 50 μ V, 5 mV, 20 ms in (C); 20 μ V, 5 mV, 500 ms in (D), E; 5 mV, 20 ms in (F).

Activity of secondary neurones

Most of the more than 100 secondary neurones impaled with KCl electrodes had resting potentials in the range 20–60 mV. Those with less than 25 mV were not studied. Neurones with 30–60 mV resting potentials had spikes from 10–40 mV in amplitude and recordings could be made for 5–15 min. Usually, they did not give an injury spike discharge upon impalement. The small size of the APs and the lack of a significant injury discharge in secondary neurones contrasted sharply with other types of cells of about the same size in the retina (see following paper; Jacklet & Rolerson, 1982), which often gave large overshooting APs and injury discharge when first impaled.

Action potentials in secondary neurones occurred during CAP activity in the optic nerve. In Fig. 6A a small depolarization occurred in the neurone just prior to the larger AP and coincident with the negative peak of the CAP. Upon light stimulation (Fig. 6B), the secondary neurone was depolarized and APs were evoked, which correlated temporally with each of the evoked CAPs.

Another secondary neurone, Fig. 6D, fired a single AP at each CAP and when stimulated by light (Fig. 6E) this neuron fired a single AP in perfect synchrony with each CAP. Each of the neurones in Fig. 6 was activated by electrical stimulation of the optic nerve (Fig. 6C, F), resulting in a response for each stimulus above threshold. In each case the larger amplitude, longer duration response provoked by the larger stimulus, which should excite more optic nerve axons, suggests there are multiple inputs to the neurones.

The secondary neurone APs evoked by light pulses sometimes had depolarizations contributing to their rise (Fig. 6B) but others did not. Both situations are seen in

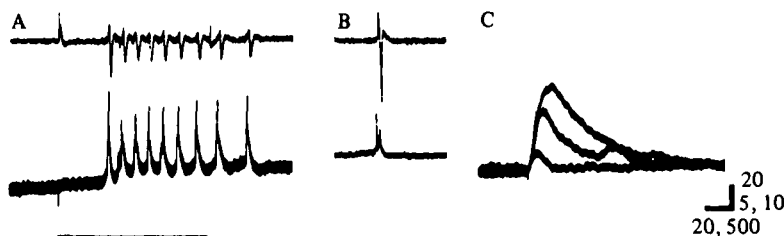


Fig. 7. Secondary neuron and optic nerve activities. Light-induced, perfectly correlated APs and CAPs (top) are shown in 7A and spontaneous dark activity is shown in 7B (resting potential 35 mV). Responses to optic nerve stimulation at 15 V, 18 and 25 V, 0.5 ms are shown in 7C. Secondary depolarizations occurred at 18 and 25 V; 25 V gave the maximum response. Scales: 20 μ V (top); 5 mV and 500 ms in 7A; 7B same except 10 mV (bottom); 7C, 5 mV, 20 ms.

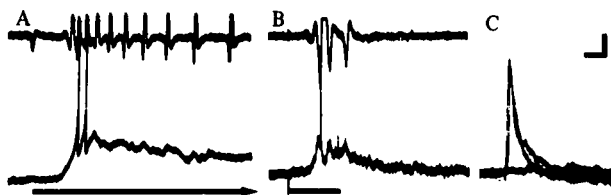


Fig. 8. Secondary neurons and optic nerve activities. The response to a prolonged light pulse is shown in 8A. The graded depolarization persisted for the entire time (not shown) but only 2 large APs occurred although small potentials are correlated with many of the CAPs. Resting potential is 60 mV. Another neurone responded to light (8B) and was invaded by antidromic spikes (8C) upon optic nerve stimulation at 10, 20, 25 V, 0.5 ms; resting potential 40 mV. Scales: 20 μ V (top), 10 mV and 500 ms for 8A; 20 μ V, 5 mV and 500 ms for 8B; 5 mV, 50 ms for 8C.

Fig. 7A where the first evoked AP is large and rises smoothly from the underlying graded depolarization. Subsequent APs have smaller potentials either just preceding or just following the main AP. The smaller potentials were most obvious when the neuron was firing at rates greater than 2 Hz and the amplitude of the main AP was diminished. Note that the reduction in amplitude of the secondary neurone AP was reflected in a reduction in the CAP amplitude. This neurone had two small APs during spontaneous firing (Fig. 7B) but unlike Fig. 6A, the larger, rapid AP preceded the smaller, slow one. Stimulation of the optic nerve evoked a compound potential in the neurone (Fig. 7C). It was graded with stimulus voltage, increasing in amplitude and duration with higher stimulus voltage.

Some neurones fired in incomplete correspondence with the optic nerve CAPs evoked by light as shown in Fig. 8A. Light evoked a train of CAPs and the neurone fired large APs (40 mV) only twice but each one was perfectly correlated with a CAP. Most of the other CAPs were accompanied by a small depolarization in the intracellular record, as though the neurone was receiving input from other cells corresponding to each CAP. The underlying slow depolarization provoked by the light persisted as long (20 s) as the stimulus continued. Incomplete correspondence between

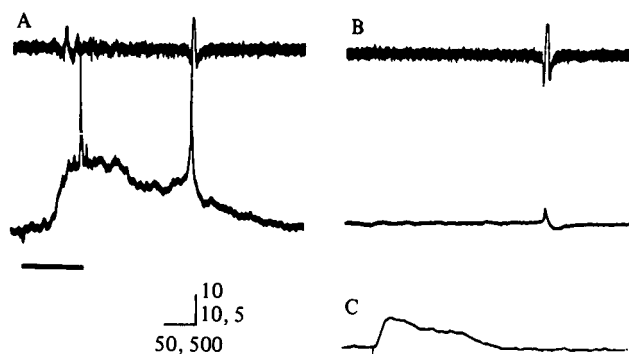


Fig. 9. Secondary neurone and optic nerve activities. Depolarizing response with large APs evoked by a light pulse (dark bar) is shown with correlated and uncorrelated optic nerve CAPs (top) (9A). Small biphasic potential is correlated with spontaneous dark CAP (10B). Stimulation of optic nerve (50 V, 0.5 msec) evoked potential (9C). Scales: 10 μ V, 5 mV, 500 ms in (9A) 10 μ V, 10 mV, 500 ms in (9B) 10 mV, 50 ms in (9C).

CAPs and intracellular APs is also shown in Fig. 8B. A large AP arose from a complex depolarizing potential on the first CAP. Subsequent CAPs were accompanied by only small depolarizing potentials. Optic nerve stimulation evoked the antidromic APs seen in Fig. 8C. They were as large as the evoked AP in Fig. 8B and graded in duration with stimulus voltage.

The activities in Fig. 8 show that not all neurones fired APs 1:1 with light evoked CAPs. Another example is shown in Fig. 9. This cell responded to illumination with a graded depolarization and APs were evoked. Each CAP was correlated with an AP except the first one, which had only several bumps riding on the graded depolarization. During spontaneous dark activity (Fig. 9B) the neurone fired only small biphasic potentials. Optic nerve stimulation produced a low amplitude (similar to 9B) depolarizing potential of long duration (Fig. 9C). The spontaneously occurring biphasic potential correlated with the CAP also occurred in R photoreceptor impaled in the axon (see following paper; Jacklet & Rolerson, 1982).

The possible involvement of chemical synapses in generating the spontaneous dark activity and light evoked responses was investigated by recording in low Ca^{2+} -high Mg^{2+} ASW, after eyes had been exposed to this medium for 1 h to ensure that the Ca^{2+} level of the neuronal environment had been lowered for a sufficient period. Known chemical synapses in *Aplysia* CNS (Halstead & Jacklet, 1974) are completely suppressed only after 20 min or so of treatment. The low Ca^{2+} -high Mg^{2+} speeded up the spontaneous dark CAP frequency as previously observed (Jacklet, 1973*b*) and the firing evoked in secondary neurones by a short light pulse was not blocked but prolonged and increased in rate (Fig. 10). The firing of the secondary neurone did not follow the frequency of CAP firing above 2–3 Hz, as shown in other figures also. These results indicate that chemical synapses are not essential for APs in secondary neurones, the depolarizations leading to APs (see late responses in Fig. 10B), or the graded depolarizations. They suggest the coupling to other retinal cells is by electrotonic synapses.



Fig. 10. Secondary neurone and optic nerve activities after 1 h in low Ca^{2+} -high Mg^{2+} ASW. Response to a 8 s pulse of light in 10A and later to a 1 s pulse is shown in 10B. Neurone had spontaneous dark CAPs correlated 1:1 with APs (not shown). Resting potential is 45 mV. Prolonged firing after light pulse (10B) is typical of responses in low Ca^{2+} -high Mg^{2+} ASW. Scales: 10 μV , 10 mV for (10A, 10B), 2 S in 10A and 500 ms in 11B.

DISCUSSION

Secondary neurones and receptors appear to have axons in the optic nerve, as shown by backfilling with cobalt in this study and Procion yellow in a previous study (Jacklet, 1976). The small neurones ($< 5 \mu\text{m}$ dia.) that were filled may be the lower retinal neurones (Strumwasser *et al.* 1979). They might have axons in the optic nerve, but may have been filled by cobalt migration across gap junction (Politoff, Pappas & Bennett, 1974).

Secondary neurones always had depolarizing potentials correlated 1:1 with spontaneous dark CAP activity. A shallow pacemaker potential of about 5 mV preceded the depolarization. The small depolarizing potentials that were observed were sometimes biphasic with a shallow hyperpolarization following the depolarization (Fig. 9B). This type of potential might be expected of an electrical junction that filters out high frequency but allows low frequency changes, such as AP repolarization in an electrically coupled neighbour neurone, to pass with less attenuation. They did not appear to result from chemical synapses since they were unaffected by low Ca^{2+} -high Mg^{2+} saline.

Electrical stimulation of the optic nerve provoked depolarizing potentials and/or small APs in secondary neurones similar to those seen during spontaneous dark and light-evoked activity. The amplitude and duration of the smaller potentials could always be increased with increasing stimulus voltage showing that the neurones receive many inputs and not just an antidromic AP. The inputs might be coming from electrically coupled secondary neurones or photoreceptors, or by chemical synapses known to exist in the retina from morphological studies. Stimulation of the optic nerve causes antidromic activity in afferent fibres from the retina but it would also cause orthodromic activity in known efferent fibres from neurones in the cerebral ganglion (Luborsky-Moore & Jacklet, 1976). These efferent fibres suppress the spontaneous CAP activity and modulate the waveform of the circadian rhythm (Eskin, 1971). Some are activated by photoreceptors in the rhinophores (Jacklet, 1980b). Since the influence of the efferent fibres is to suppress ongoing CAP activity,

it is interesting that inhibitory potentials were not observed in secondary neurones in response to optic nerve stimulation.

The soma and the dendrites appear to receive the electrotonic inputs from other cells, judged by the morphological studies (Strumwasser *et al.* 1979) showing gap junctions between secondary neurones and between photoreceptors containing small, clear, cytoplasmic vesicles and secondary neurones (Luborsky-Moore & Jacklet, 1976). These gap junctions may be the pathway for the Lucifer yellow dye coupling observed among secondary neurones and between secondary neurones and photoreceptors. Lucifer yellow dye coupling via gap junctions has been observed among neurosecretory bag cells of *Aplysia* (Kaczmarek *et al.* 1979). The weak electrical coupling among the bag cells allows them to fire synchronously. The graded depolarization of the secondary neurones in response to light may come via gap junction from receptors, since it is not suppressed by low Ca^{2+} -high Mg^{2+} ASW, and so is not mediated by a chemical synapse. It seems unlikely that the secondary neurones respond to light directly because they have no morphological specialization for light reception. Additional evidence for the depolarization coming from photoreceptors is the dye coupling observed between photoreceptors and secondary neurones (Fig. 5).

The small size of the APs (10–20 mV for spontaneous, 10–40 mV for light evoked) in secondary neurones suggests that the soma is not capable of generating an AP. Therefore, they are being initiated at some distance from the point of recording, which was presumably the soma. Even the larger APs (from neurones with 60 mV resting potentials) recorded in response to light did not overshoot the zero potential.

Depolarization of secondary neurones with applied current failed to evoke APs in the secondary neurones or to provoke CAPs. However, this was only attempted on a few occasions and insufficient current may have been used. If the secondary neurones are extensively coupled to one another and other cells by gap junction, one would expect the input resistance of these neurones to be quite low and therefore less responsive to applied currents. Photoreceptors that spiked (see following paper, Jacklet & Rolerson, 1982) could easily be depolarized to produce APs.

This study indicates that the secondary neurones are organized as follows. The secondary neurones are endogenously active and weakly electrical coupled. The endogenous activity of the secondary neurones is controlled by the circadian clock, which appears to be a property of the neurones or the electrically coupled photoreceptors. Chemical synapses are not needed for secondary neurone activity, as shown in this study, or the circadian rhythm (Jacklet, 1973*b*) and therefore a model previously advanced (Audesirk, 1973; Strumwasser, 1973) for the eye organization that required chemical synapses from pacemaker neurones to secondary neurones is incorrect. Slow changes in potential spread easily through the population of neurones and many neurones reach the threshold for firing together resulting in the synchronous firing of secondary neurones, seen in the optic nerve as a CAP. Each secondary neurone has an axon in the optic nerve. Not all secondary neurones contribute to each CAP since the size of a CAP may vary considerably. Light evoked depolarizations of secondary neurones are mediated by electrotonic synapses from photoreceptors to secondary neurones. The photoreceptors involved are most likely those with large microvilli, and small, clear vesicles in their cytoplasm, which give a large graded depolarization in

response to light. These photoreceptors and others are examined in the following paper (Jacklet & Rolerson, 1982).

Eyes of three other gastropods (*Navanax*, Eskin & Harcombe, 1977; *Bursatella*, Block & Roberts, 1981; and *Bulla*, Block & Friesen, 1981) are known to display circadian rhythms of spontaneous dark CAP activity. The rhythms are associated with the frequency of CAP activity in the optic nerve and not with the unsynchronized unit activity. Therefore, each eye displaying a rhythm would be expected to have a population of synchronously firing neurons responsible for the CAP, like the secondary neurones of *Aplysia*. Secondary neurones are seen in histological sections of the *Bulla* eye (Jacklet, unpublished).

Other simple gastropod eyes that contain secondary neurones (*Helix*, Brandenburger, 1975; *Littorina*, Newell, 1965; and *Lymnaea*, Stoll, 1973) have not been tested for circadian rhythm. Some gastropod eyes may not have secondary neurones in the retina. An example is the relatively advanced, optically-useful eye of *Strombus* (Gillary & Gillary, 1979). The eye of *Hermisenda* has an optic ganglion containing fourteen secondary neurones separate from the retina. They are spontaneously active in darkness and are hyperpolarized in light via chemical synapses from photoreceptors (Alkon, 1973), unlike *Aplysia* secondary neurones.

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