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THE CRAYFISH VISUAL SYSTEM:
INTRACELLULAR STUDIES AND MORPHOLOGIES
OF IDENTIFIED INTERNEURONS

by

Mark D. Kirk

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

DOCTOR OF PHILOSOPHY

APPROVED, THESIS COMMITTEE

Dr. Raymon Glantz
Professor of Biology
Chairman

Dr. Ronald Sass
Professor of Biology

Dr. John Clark
Professor of Electrical Engineering

HOUSTON, TEXAS

APRIL 1982
ABSTRACT

THE CRAYFISH VISUAL SYSTEM:
INTRACELLULAR STUDIES AND MORPHOLOGIES
OF IDENTIFIED INTERNEURONS

By Mark D. Kirk

A semi-intact eye-cup preparation was developed which maintains visual responses and enables intracellular recordings to be made from the optic lobes of the crayfish compound eye. Sustaining fibers (SFs) were impaled with lucifer yellow electrodes near their entrance to the second optic ganglion or Medulla (where the SFs originate). Corneal receptive fields were determined and the SFs identified based on the previous work of Wiersma and Yamaguchi (1966). Following identification of each cell, the dendritic morphology was observed with lucifer yellow iontophoresis and subsequent fluorescence microscopy. An individual SF possesses a dendritic arborization restricted to that portion of the Medulla corresponding to its corneal receptive field. Therefore, the position of the dendritic tree combined with the retinotopic organization within the distal optic neuropils determine each SF's visual receptive field.

Contour sensitivity maps were obtained for SF 019. The quantitative variation in sensitivity along a given arc in visual space was correlated with 019 dendritic density along the corresponding portion of the Medulla.

The SFs' responses to various stimulus conditions were
recorded intracellularly in their integrating segments. No synaptic interactions between SFs were observed. Bursting in response to broad-field intense illumination results from periodic excitatory synaptic input.

Other visual interneurons were impaled and dye-filled. These included, a) Phasic light-on cells, b) Tonic light-off cells, c) Phasic light-on and -off cells, d) Optic tract motion detectors, e) Nonspiking light-on neurons, f) Lamina monopolar cells, and g) Lamina tangential cells (TAN2).

A group of neurosecretory cells analogous to the Medulla Externa X-Organ of other crustaceans was discovered in the crayfish. These neurosecretory cells are also interneurons possessing a modality specific (visual) synaptic input. They are inhibited by step increases in illumination and the response lasts for the duration of the stimulus. Transient off-inhibition is commonly present. The IPSPs reverse near resting membrane potential and appear to be GABA mediated and chloride-dependent. These cells project axons to the proximal edge of the Lamina and have a vertical plane of dendrites in the same layer of the Medulla as the SFs. It is proposed that these cells are involved in the neurosecretory control of the circadian rhythm of screening pigment migration and/or photoreceptor sensitivity in the retina.
ACKNOWLEDGMENTS

This thesis is dedicated to the memory of a dear friend and colleague Duane R. Ringer. May his spirit of sincerity and good will dominate our remembrance of him.

There are many others whose support, guidance and friendship are greatly appreciated. These include Raymon Glantz, Brian and Deb Waldrop, Harvey Nudelman, Colette Marcelin, Mary LeBus, Roar Irgens, Maelane Rhodes, Shirley Smith and of course Mr. and Mrs. Carl L. Kirk who deserve a degree of their own.

I owe special thanks to Raymon Glantz and Brian Waldrop for directly participating in some of the experiments reported in this thesis. This research was supported by a N.I.H. predoctoral training award (Grant EY-07024-03) and a N.S.F. research grant (BNS 79-10335).

M.D.K.

April, 1982
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INTRODUCTION AND OVERVIEW OF THE
CRAYFISH VISUAL SYSTEM

Visual systems are attractive to work with for a number of reasons. The effective stimulus energy (light) is very easy to deliver to the preparation and to quantify (photons cm\(^{-2}\)sec\(^{-1}\)). In most cases the nervous elements involved are arranged in parallel and in series, with abundant lateral interactions. We, therefore, have the opportunity to study networks of neurons in addition to cellular properties. The peripheral visual systems of most animals are at least in part embryonically derived from primordial central nervous tissues. It is assumed, therefore, that observations obtained in visual systems are directly applicable to operations that take place within the central nervous system (CNS = brain or spinal cord). I have studied the crayfish visual system in order to further our understanding of peripheral vision in this animal and to relate these findings to central nervous structures of all arthropods and, if possible, higher animals in general.

Crayfish are stalk-eyed crustaceans. Their compound eyes are supported by a flexible extension of a rigid chitinous eye-cup that surrounds a multi-faceted cornea. The eye is of the superposition type (Bullock and Horridge, 1965), so it is well designed for a nocturnal habit. During the night (or when dark-adapted) the visual screening pigments surrounding (and within) the photoreceptors are retracted so that a maximum amount of incident light is absorbed by the
photopigment. Under constant illumination (light adaptation),
the screening pigments disperse, optically isolating adjacent
ommatidia and the corresponding photoreceptors. This, how-
ever, is a condition characteristic of apposition compound
eyes which are well adapted for diurnal habits. Therefore,
the crayfish is capable of adjusting its eyes from a high
sensitivity, low resolution (superposition) condition during
the night to a low sensitivity, high resolution (apposition)
state during the day (Land, 1979).

Proximal to the retina, hidden within the eye-cup, are
a series of four optic ganglia responsible for the neural
processing of visual stimuli (Parker, 1895; Wiersma and
Roach, 1977). They are traditionally numbered 1-4, from most
distal (nearer the retina) to most proximal (nearer the long-
itudinal midline of the animal's body) as follows (Fig. 1B)\(^1\):

First Optic Ganglion = Lamina (LG) (Lamina Gangli-
onaris)

Second Optic Ganglion = Medulla (M) (Medulla Ex-
terna)

Third Optic Ganglion = Lobula (L) (Medulla Interna)

Fourth Optic Ganglion = Lateral Protocerebrum (LP)
(Medulla Terminalis)

The four optic ganglia have been thought to contain, re-
spectively, the second, third, fourth, and fifth order inter-
neurons of the crayfish visual system; the first order cells

\(^1\)Old terminology in parentheses; new terminology according
to Strausfeld and Nassel (1981).
being the primary photoreceptors of the retina (Parker, 1895).

The insect compound eye possesses many similarities and
direct analogies with that of the crustacean (Bullock and
Horridge, 1965; Strausfeld and Nassel, 1981), and extensive
studies have been published on the anatomical and physiolog-
ical substrates of visual activity within the peripheral ele-
ments of both the insect compound eye as well as the simple
ocelli (for reviews see Kirschfeld, 1979, and Shaw, 1979,
1981). However, the literature of crustacea has been for the
most part restricted to studies on the photoreceptors and
their associated optical apparatus (see Waterman, 1981) and
the larger elements present within the optic tract (OT),
which connects the LP and the brain (Supraesophageal Gang-
lion) (Wiersma, et al., 1982). An exception is the ocellus of
the barnacle (Laughlin, 1981; Oertel and Stuart, 1981; Stuart
and Oertel, 1978).

A. THE ARTHROPOD LAMINA (LG)

1. MORPHOLOGY

A great deal of histology has been performed on the ar-
thropod Lamina, including Golgi-Electron Microscopy (Golgi-
EM). This technique has enabled the determination of many
cell types in the insect Lamina and the morphological con-
nectivity patterns associated with them (Strausfeld, 1976).
The most recent detailed synopsis of these connections ap-
ppears in a review by Shaw (1981). Golgi and EM have been
performed on the decapod Lamina (Hamori and Horridge, 1966; Hafner, 1973; Nassel, 1977; Stowe, et al., 1977). It is now well established that an unpermutated retinotopic projection exists from the retina into the Lamina of the crab and crayfish (Stowe, 1977; Strausfeld and Nassel, 1981). An orderly 1:1 mapping occurs from each set of photoreceptors within an individual ommatidium onto the corresponding neurommatidium (or optic cartridge) in the Lamina. This retinotopic mosaic continues through the columnar organization of the Medulla and Lobula (see Sect. B).

In the crayfish all eight photoreceptors (R1-R8) of a given ommatidium subtend the same visual angle (have the same visual field), and for the most part neighboring ommatidia of light-adapted eyes are optically isolated (Shaw, 1969; Walcott, 1974). As a result a particular portion of visual space can theoretically be traced through the first three optic ganglia.

The synaptic connectivity between crayfish photoreceptors and Lamina neurons was studied by Nassel and Waterman (1977). However, the description of synaptic interconnections within the Lamina is in its infancy (Nassel, 1977; Strausfeld and Nassel, 1981). It should be noted that another crustacean with a simpler Lamina organization has been extensively studied with serial EM (Macagno, et al., 1973; Nassel, et al., 1978), and the synaptic connectivity is well established. Briefly, I will describe what is known of the crayfish Lamina and point out possible analogies with that of
the insects. The most detailed description of a crayfish Lamina is of the species *Pacifastacus leniusculus* (Nassel, 1976, 1977; Strausfeld and Nassel, 1981). The crayfish Lamina contains five anatomicallayers, each containing unique portions of identifiable neurons. From distal to proximal they are:

a) Outer cell body layer (OCL)
b) Inner cell body layer (ICL)
c) Outer plexiform layer (epl₁)
d) Inner plexiform layer (epl₂)
e) Proximal cell body layer (pcl)

Contributing processes to two or more of these layers are the following cell types:

a) Photoreceptor axon terminals (R1-R8)
b) Lamina monopolar cells (M1-M5 or LMCs)
c) Morphological centrifugal cells (CI)
d) Tangential cells (TAN1 and TAN2)
e) Multipolar cells (MP1 and MP2)

Each of these cell types has a counterpart in the insects (Shaw, 1979). The LMCs are thought to be the major carriers of visual signals from the Lamina to the Medulla. They do so in the insects by passive propagation along their axons, although considerable controversy still exists over this point (Shaw, 1979). The LMC axons make an anterior-posterior crossing between the Lamina and Medulla. This horizontal reversal, or chiasma, is also present in the crayfish and consequently visual space was thought to be
represented in a reversed manner within the Medulla (Parker, 1895). The major portion of photoreceptor input to the LMCs is from the seven short visual fibres, R1-R7, which are yellow-green sensitive (Goldsmith, 1978; Nosaki, 1969; Waterman and Fernandez, 1970). The eighth retinular cell (R8) projects in parallel with the LMCs through the chiasma apparently synaptically bypassing the Lamina to terminate in the Medulla (Nassel, 1976). R8 provides the optic tract interneurons with a blue-violet sensitivity (Cummins and Goldsmith, 1981; Trevino and Larimer, 1970; Woodcock and Goldsmith, 1970).

2. PHYSIOLOGY

Most of what we know about the intracellular responses of Lamina cells to light stimuli comes from reports on the insects (Laughlin, 1981; Shaw, 1981). This includes the pioneering study by Shaw (1968) in the locust, followed by works on the fly (Autrum, et al., 1970) and the dragonfly (Laughlin, 1973). These are restricted to recordings from photoreceptor terminals and the LMC axons, although a single recording from a morphological centrifugal cell has been reported (Jarvilehto and Zettler, 1973). Arthropod photoreceptors depolarize in response to light-on while LMCs exhibit a maintained hyperpolarization to this stimulus. Therefore, the first visual synapse appears to be inverting and chemical (Laughlin, 1981; Shaw, 1979).

A number of methods have been developed and applied
through the use of intracellular recording in order to characterize Lamina cells physiologically. In the following, I will discuss the relevant characteristics of insect LMCs (for a review see Laughlin, 1981):

a) LMCs have a low resting membrane potential (RMP) and hyperpolarize to light-on (Jarvilehto and Zettler, 1971; Laughlin, 1973). The waveform of the LMC response, however, depends on the stimulating light intensity. At low light levels a pulse of incident light leads to a noisy but pure hyperpolarization. As light intensity is increased the waveform becomes triphasic with an initial transient hyperpolarization followed by a plateau (sometimes depolarized with respect to RMP) and finally a depolarizing response subsequent to light-off. A single recording from L4 in the fly indicates a spiking on-response similar to the spiking cells reported by Arnett (1971) (Hardie, 1978 cited by Laughlin, 1981).

b) Latency measurements in LMCs when compared to those of photoreceptor terminals, for a range of light intensities, have led to an estimated synaptic delay of 1-2 msec (Jarvilehto and Zettler, 1971; Laughlin, 1973; French and Jarvilehto, 1978). This is consistent with the monosynaptic connections between photoreceptors and LMCs seen histologically (Strausfeld and Campos-Ortega, 1977).
c) The angle of acceptance sensitivity function for LMCs is contracted when compared with that of the presynaptic retinular cells, R1-R6. This result in the fly was what first led Zettler and Jarvilehto (1972) to suggest lateral inhibition existed within the Lamina. More recently Shaw (1981) has presented direct evidence of an opposing influence from neighboring pathways in the Lamina.

d) A plot of intracellular response magnitudes to a series of calibrated light intensities is known as the response/intensity (Vm/logI) function for that cell type. Arthropod photoreceptors (crayfish included, Glantz, 1968) have a wide range of sensitivity to light with Vm/logI functions which span 3 to 5 log units of light intensity (Laughlin, 1981). However, the LMC peak and plateau response saturate within a range of 2 log units of light intensity. The slope of the on transient \( \frac{dV_m}{dt} \) increases monotonically over 5 log units of light intensity, and Jarvilehto and Zettler (1971) suggested that the slope of the on transient may be the only accurate representation of light intensity at this level. However, Laughlin and Hardie (1978) demonstrated that adaptation mechanisms prevent LMC saturation during intense background illumination, making unnecessary an a priori argument for coding by rate of change (Laughlin, 1981).
e) Membrane resistance changes during LMC light responses have been reported using a single electrode and bridge circuit (Shaw, 1968; Laughlin, 1974). Although subject to large measurement errors (due to possible electrode resistance changes) a decrease in resistance does occur in the LMCs during light activation. The same result has been more carefully documented in the locust ocellar L-neurons (Wilson, 1978) and barnacle ocellar I-neurons (Oertel and Stuart, 1981).

f) The LMCs exhibit little or no differential sensitivity to the wavelength or polarization of light (Zettler and Autrum, 1975; Laughlin, 1976), apparently due to the convergence upon individual LMCs by photoreceptors of combined or antagonistic sensitivities (wavelength and polarization of e-vector, Laughlin, 1976; Zettler and Autrum, 1975).

None of the above characterizations (a-f) have been performed on the Lamina of any crustacean compound eye. The only published recordings to date are from an unidentified cell of the crab Lamina (Erber and Sandeman, 1976). Although the responses appear similar to the insect LMCs no quantitative description of any of the above parameters were reported, only a cursory example of the effect of increasing light intensity.

The barnacle (Crustacea, Cirripedia) simple ocelli have been studied extensively (Ozawa, et al., 1975), and many
similarities appear when compared to the insect ocelli and compound eye. The second order cells (I-neurons) have a triphasic predominately hyperpolarizing response to a pulse of light, but both the peak and plateau phases are graded over 5 log units of light intensity (Stuart and Oertel, 1978). The I-cells passively propagate the visual signal to third order cells (A-neurons) that spike in response to light-off. Therefore, the second visual synapse is excitatory and chemical (Oertel and Stuart, 1981).

B. THE ARTHROPOD MEDULLA (M)

1. MORPHOLOGY

Prior to the present study the arthropod Medulla was an enigma (Kirk, et al., 1982). What was known about the morphology of the Medulla came from golgi studies in the insects (Campos-Ortega and Strausfeld, 1972; Strausfeld, 1976) and a few crustaceans (Strausfeld and Nassel, 1981). Strausfeld and Nassel (1981) published a summary of cell types and connectivity patterns where known in the crayfish optic ganglia. Included was the observation that the retinotopic organization between the Retina and Lamina is continued through the Medulla and Lobula. Therefore, every cartridge within the Lamina projects to a corresponding column in the Medulla following an 180° horizontal reversal at the first optic chiasma. This result was an important precedent for the present study (Kirk and Glantz, 1981a; Kirk, et al., 1982).

Strausfeld and Nassel (1981) also describe the layering
of the Medulla and the neuronal types distinguishable based upon their unique morphologies. In one case it was possible to ascribe dopamine as the neurotransmitter for a particular morphological cell type (Elofsson et al., 1977). To my knowledge, there has only been a single report of electron microscopy performed on the medullary neuropil (Shivers, 1967). No attempt was made to relate synaptic profiles to morphological cell types except for Type I and II neurosecretory cells (see Sect. IV, A).

2. PHYSIOLOGY

Reports of extracellular recordings from the insect Medulla are common (Adrian, 1937; Bishop et al., 1968; Honneger, 1980; Wiersma and Roach, 1977). Characteristically these neurons respond to light-on and exhibit motion sensitivity which is not direction specific.

Recently intracellular recordings and dye-fillings have appeared of these cells in the fly (DeVoe and Ockelford, 1976) and bee (Hertel, 1980). However, these studies added little to our understanding of the structure-function relationships at the cellular level within the Medulla. DeVoe (1980) recorded from nonspiking neurons which depolarized in response to increased illumination. He argued that these were the transmedullary neurons (present in each column) which carry the retinotopic mosaic into the Lobula. Although this is a reasonable interpretation, no dye injections were presented and the morphological cell type with the above response
properties remains to be established.

Until the recent studies in this laboratory only two cases of recordings from the crustacean Medulla had been published (Erber and Sandeman, 1976; Leggett, 1976). These were single unit extracellular responses from cells in the crabs Leptograpsus and Scylla. In both cases the recordings were from neurons which responded maximally to light-off. All other published investigations of visual interneurons of the decapod optic ganglia utilized extracellular recordings from axons in the optic tract (i.e., optic nerve) (Arechiga and Yanagisawa, 1973; Glantz, 1971; 1973; Glantz and Nudelman, 1976; Wiersma and Yamaguchi, 1966; 1967; Wiersma et al., 1982; Yamaguchi and Otsuka, 1973). The notable visual cell types found in the crayfish optic tract include sustaining fibers (SFs) (i.e., tonic light-on units), dimming fibers (i.e., tonic light-off units) and jittery motion detectors. In the present study I concentrated on the SFs. A review of their relevant characteristics deduced entirely from extracellular axonal recordings is as follows:

Crayfish SFs are visual interneurons with large, well defined corneal receptive fields (Wiersma and Yamaguchi, 1966). The SFs are excited by a step increase in illumination. The response consists of a transient followed by a tonic firing which lasts for the duration of the stimulus and both are proportional to the log of the light intensity (Glantz, 1971). Wiersma and Yamaguchi (1966) first recorded from SF axons and identified 14 separate cells in each optic
tract based on their unique receptive fields.

Glantz (1971) has compared the waveform of the $V_m$/log$I$ functions (obtained from light pulses) of retinular cells and SFs. The SF response was found to decay much more rapidly than the photoreceptor's. In addition, the SF's on-transient was thirty times more sensitive (on the absolute intensity scale) than the corresponding portion of the retinular cell response. He also found that when measured one second after the onset of a light step (i.e., the tonic response) the retinular cell was still sensitive over 4 log units while the SF saturated (at 15% peak rate) within 2 log units of light intensity. He concluded that "... little information regarding the ambient light intensity is passed along the optic nerve... however, the sustaining fibre maintains a large transient dynamic range permitting a wide variation of response amplitude to incremental stimuli."

Each SF exhibits lateral inhibition from all areas of the cornea surrounding its receptive field (Arechiga and Yanagisawa, 1973; Wiersma and Yamaguchi, 1967). Some may also possess a form of inhibition within the receptive field (Glantz, 1973; Wiersma and Yamaguchi, 1967). Arechiga and Yanagisawa (1973; see also Wiersma and Yamaguchi, 1967) concluded that the SF off response emerges from a form of lateral inhibition. Glantz and Nudelman (1976) have proposed further that a lateral inhibitory network is responsible for the periodic SF bursting conspicuous during broad field illumination. They suggested that the network probably does
not include the SFs but merely imposes its output upon them.

SF spectral sensitivity functions indicate inputs from both the long wavelength (\(\lambda_{\text{max}} = 570\text{nm}\)) and short wavelength (\(\lambda_{\text{max}} = 450\text{nm}\)) pathways (Trevino and Larimer, 1970; Woodcock and Goldsmith, 1970; 1973). These peak wavelength sensitivities correspond to those of the yellow-green and blue photoreceptors found in the retina (Nosaki, 1969; Waterman and Fernandez, 1970).

Contour sensitivity maps of SF receptive fields indicate a \(10^\circ - 30^\circ\) region of uniform high sensitivity falling off by as much as 0.5 log unit for a \(10^\circ\) step into the visual surround (Glantz, 1973; Woodcock and Goldsmith, 1973; and this study).

With respect to the functional outputs of the SFs, we know that they possess efferent processes in the Medulla Terminalis (or Lateral Protocerebrum), and Supraesophageal Ganglion, the crayfish brain (this study and Waldrop and Glantz, 1980). The brain terminals make monosynaptic contact with eye muscle motoneurons (Nudelman and Glantz, in preparation), and they also excite tonic-on interneurons which enter the circumesophageal connectives (Glantz and Wood, 1978). The postsynaptic targets of the Lateral Pro- tecerebral terminals are unknown. (see Sect. I, D)
I. SUSTAINING FIBER MORPHOLOGY AND RECEPTIVE FIELD-DENDRITIC FIELD CORRELATION

A. INTRODUCTION

Until the recent studies in this laboratory (Kirk and Glantz, 1981a; Kirk et al., 1982; Waldrop and Glantz, 1980) no successful attempt to bridge the gap between our knowledge of the optic tract interneurons and the optic ganglia had been reported. In this section I will describe the general morphology of SFs, the specific (and unique) morphologies of several identified SFs along with their variability, and attempt to relate these findings to their relevant visual physiology.

B. MATERIALS AND METHODS

Male or female crayfish (Pacifastacus leniusculus) measuring 8-11 cm from rostrum to telson were obtained from Monterey Bay Hydroculture Farms, Santa Cruz, California. Several Procambarus clarkii yielded similar results.

A semi-intact eye-cup preparation was developed for this study (Fig. 1A). The rostrum and chelipeds were removed and the wounds allowed to heal. The eyestalks were glued to the cephalic carapace with the corneas directed laterally. The animal was slowly cooled to 5°C then placed in a container of continuously oxygenated crayfish saline (Van Harreveld, 1936) on ice. The pericardial cavity and ventral abdominal sinus were opened to allow the blood to exchange with the saline. After one hour the crayfish was transferred to a plexiglass dish (along with the oxygenated saline) and secured dorsal
side up. The optic lobes of the right eye were immediately exposed and desheathed. Prior to positioning the electrodes the heart was surgically removed. This allowed stable intracellular recordings within the optic lobes by preventing blood pulsation. Although previous reports have noted the extreme sensitivity of the decapod visual system to anoxia (Waterman et al., 1964; Taylor, 1974; Ache and Sandeman, 1980), with the procedure described above visual interneuron responses remained healthy for several hours, comparable to extracellular recordings from intact animals (Wiersma and Yamaguchi, 1966).

The SFs were impaled near the proximal edge of the Medulla with beveled (Ogden et al., 1978) Lucifer Yellow-CH filled micro-pipettes (3% W/V, tip diameters 0.5 μm; courtesy of Walter Stewart, Stewart, 1978) using standard electrophysiological techniques. Simultaneous extracellular recordings were made with tungsten electrodes placed in the optic tract near its entrance to the supraesophageal ganglion (brain, Fig. 1A).

The corneal receptive fields of impaled SFs were mapped with a small (≈1 mm diameter) hand-held light spot as described previously (Wiersma and Yamaguchi, 1966). Each cell was then identified and named according to Wiersma and Yamaguchi (1966). During the identification procedure the animals were moderately light-adapted. The results presented here are from 34 SFs (representing 9 of those 14 identified previously by Wiersma and Yamaguchi, 1966), whose corneal
FIGURE 1

THE SEMI-INTACT EYE-CUP PREPARATION
AND ORGANIZATION OF THE
CRAYFISH COMPOUND EYE

A. Schematic of the preparation viewed frontally illustrating the intracellular (INT) and extracellular (EXT) sites of recording. Abbreviations: A, antennules; C, cornea; OL, optic lobes; R, rostrum; SG, supraesophageal ganglion.

B. General arrangement of the crayfish right eye and optic lobes viewed from a dorsal aspect. The solid arrows indicate visual space orientation within the Retina, Lamina and Medulla. Abbreviations: C, Cornea; R, Retina; L.G., Lamina Ganglionaris; M, Medulla (Medulla Externa); L, Lobula (Medulla Interna); O.F., Optic Foci (Medulla Terminalis); O.T., Optic Tract (old terminology in parentheses, new terminology according to Strausfeld and Nassel, 1981).
receptive fields were determined and dendritic projections examined by subsequent dye injection.

Lucifer yellow was iontophoresed into the impaled SF using constant current or 0.5 sec pulses (1-10nA) at 1 Hz delivered through the intracellular electrode for 30 to 60 minutes. The optic lobes and a segment of the optic tract were then placed in 4% paraformaldehyde in 0.1M phosphate buffer (pH = 7.5). After 3 hours of fixation the tissue was dehydrated in an alcohol series, cleared in methyl salicylate, and viewed in whole mount with a fluorescence microscope (Glantz and Kirk, 1980).

Photographs were taken at successive focal planes from a minimum of two different orientations with Kodak Ektachrome 400ASA film. Drawings were made from projections of the photographic slides by tracing the SF dendrites and the outline of the Medulla.

Subsequent to photographing the whole mount the tissue was embedded overnight in paraffin (Paraplast). Horizontal sections were cut with steel knives at ten-micrometers thickness, cleared in xylene, mounted with coverslips using Fluoromount and photographed.

In order to obtain contour sensitivity maps of SF receptive fields the animal was prepared for extracellular recording as described above except the heart and ventral blood sinus were not exposed. Instead, the animal was positioned with its eye placed at the optical center of a perimeter device. The device delivered a point source of light through
a gimbal mounted tube. The tube contained an eight-millimeter fiber optic light guide, an iris diaphragm, a projecting lens and fitted piece of cardboard with a pinhole light stop. The light guide was brought to a focused quartz-iodide lamp (Sylvania 500Q/CL), an electromechanical shutter and 6 log unit neutral density wedge. Stimuli were presented as 500 msec flashes every five seconds at five-degree intervals. Spike counts were taken over the entire response and plotted as a function of position. The corresponding log intensity (Log I) was obtained from a response/intensity (No. spikes/Log I) function performed at the most sensitive part of the recorded units receptive field (Glantz, 1973).

C. RESULTS

Each SF possesses a vertical uniplanar dendritic field within the second tangential Cell layer of the Medulla (Fig. 2). This layer approximately bisects the medullary neuropil as described by Strausfeld and Nassel (1981). Most SFs have a large integrating segment which courses dorso-ventrally along the proximal aspect of the Medulla. Figure 2A is a medial view of SF O19 illustrating the general arrangement of the SF dendrites within the neuropil. The name assigned to this neuron (O19) is from the nomenclature of Wiersma and Yamaguchi (1966). The "O" stands for optic or optic tract and "19" is the number assigned to the crayfish SF with a particular and unique corneal receptive field (see below).

The SF integrating segment extends over the Lobula
FIGURE 2
SUSTAINING FIBER MORPHOLOGY:
THE DENDRITES, INTEGRATING SEGMENT
AND CELL BODY

A. Whole mount of a lucifer yellow fill of O19 illustrating the organization of SF dendrites in the Medulla (M) and integrating segment passing proximally over the Lobula (L) neuropil. Medial view, dorsal is up. Scale = 200 micrometers.

B. Low magnification of a SF whole mount as in A except viewed from a dorsal aspect (right eye). Note that the SF dendrites are confined to a single plane within the medullary neuropil. Abbreviations: M, Medulla; L, Lobula; LP, Lateral Protocerebrum. Scale = 500 micrometers.

C. A ten-micrometer horizontal section of a lucifer yellow filled SF. A portion of the dye-filled SF dendrites are visible in the second (and largest) tangential cell layer of the medullary neuropil. Abbreviations: M, Medulla; LG, Lamina Ganglionaris. Scale = 200 micrometers.

D. Another section as in C except at a more dorsal level. Here the neurite leading to the laterally placed cell body can be seen joining the integrating segment just proximal to the Medulla (M). The sinus gland (SG) is lateral to the SF tract. Scale = 200 micrometers.
tapering to its narrowest point in an average of 521 ± 128 S.D. micrometers from the edge of the Medulla (Fig. 3A). This constriction has the smallest diameter (average = 4.8 micrometers) along the SF main axis and presumably is the region where SF spike initiation takes place (Table 1).

A fine neurite leading to the cell body exits the main SF process midway between the Medulla and spike initiating zone (S.I.Z.) (Fig. 2B and D). The SF somata (mean diameter = 33 micrometers) lie in a cluster lateral to the Lobula neuropil underneath the sinus gland (Fig. 2D, 7B).

The SF main process expands again in the Lateral Protocerebrum (mean axon terminal diameter = 7.8 micrometers) before entering the optic tract (mean axon diameter = 13.6 micrometers). On several occasions glial cells were seen dye-coupled to SF axons (Fig. 3D). This suggests the long axonal projection in the optic tract requires glial support cells much as the giant axons of the ventral nerve cord (Viancour et al., 1981).

Within the Lateral Protocerebrum the SFs send one or two presumably efferent processes into the neuropil (Fig. 3B, and C). Recent horseradish peroxidase stainings of SFs suggest that these terminals are more extensive than revealed by lucifer yellow injections (Brian Waldrop, personal communication). The SF brain terminals have been described elsewhere (Waldrop and Glantz, 1980) and were not a focus of this study.

One SF (O74) deviates consistently from the above
TABLE I

SUMMARY OF SF MORPHOLOGY

(Means Diameters in Micrometers ± S.D.)

<table>
<thead>
<tr>
<th>Component</th>
<th>N</th>
<th>Mean (μ)</th>
<th>Standard Deviation (σ)</th>
</tr>
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<tbody>
<tr>
<td>Cell Body</td>
<td>22</td>
<td>33.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Integrating Segment</td>
<td>29</td>
<td>22.7</td>
<td>5.8</td>
</tr>
<tr>
<td>Axon</td>
<td>6</td>
<td>13.6</td>
<td>3.7</td>
</tr>
<tr>
<td>Neurite</td>
<td>10</td>
<td>2.9</td>
<td>0.3</td>
</tr>
<tr>
<td>S.I.Z.</td>
<td>17</td>
<td>4.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Primary Branches</td>
<td>27</td>
<td>11.0</td>
<td>3.9</td>
</tr>
<tr>
<td>Secondary Branches</td>
<td>28</td>
<td>6.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Tertiary Branches</td>
<td>29</td>
<td>2.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Axon Terminal</td>
<td>8</td>
<td>7.8</td>
<td>1.9</td>
</tr>
<tr>
<td>I.S. to S.I.Z. Length</td>
<td>13</td>
<td>521.0</td>
<td>127.8</td>
</tr>
</tbody>
</table>
FIGURE 3

SUSTAINING FIBER MORPHOLOGY:
THE SPIKE INITIATING ZONE,
LATERAL PROTOCEREBRAL TERMINALS
AND COUPLED GLIAL CELLS

A. Medial view of a lucifer yellow filled SF in whole
mount illustrating the tapering portion of the main
SF process to its narrowest point, presumably the
site of spike initiation (arrowhead). Abbreviations:
M, Medulla; L, Lobula; LP, Lateral Protocerebrum.
Scale = 500 micrometers.

B. & C. High magnification views of SF terminals in the
Lateral Protocerebrum from two lucifer yellow filled
SFS. Scale = 200 micrometers.

D. Dye-coupling to glial cells surrounding the axon in
the optic tract. The dye has concentrated in the
nuclei of the glial cells -- bright spots contiguous
with the lucifer yellow filled axon. Scale =
200 micrometers.
description having a biplanar dendritic field within the Medulla (Fig. 4B). The larger projection lies within the common SF layer whereas the more proximal dendrites are within the Serpentine Layer separating the inner one-eighth of the medullary neuropil from the outer three quarters (Strausfeld and Nassel, 1981). Sustaining fiber 074 has a well documented high threshold to light-on (Wiersma and Yamaguchi, 1966; personal observations), but it possesses no other exceptional physiological characteristic that could be correlated with the dendritic projection in the Serpentine Layer.

Figure 5 presents reconstructions of four SFs. The approximate outline of the neuropil, divided into quadrants, is superimposed on the tracings of the dendrites. Note that each SF innervates a well defined and unique portion of the Medulla.

Although the SFs appear to enter the Medulla in a common tract (dorsomedially), the higher order dendritic processes of an individual SF are restricted to that portion of the Medulla corresponding to its corneal receptive field (Fig. 6). As an example, consider SF 019 in Fig. 6. Its corneal receptive field includes most of the back (posterior) one-half of the eye. The lucifer yellow fluorescence revealed a dendritic field contained within the medial one-half of the Medulla. Therefore, the SF dendritic projection appears to define its corneal receptive field and confirms an 180° anterior-posterior reversal in visual space representation at the level of the Medulla. No change in the dorsal-ventral
FIGURE 4
TRACINGS OF SUSTAINING FIBER 074

A. Medial view of 074 with the approximate outline of the Medulla indicated by the dashes.

B. Dorsal view of 074 illustrating the unique bi-planar dendritic projection.
FIGURE 5
EXAMPLES OF SUSTAINING FIBER DENDRITIC FIELDS

Tracings of SF dendrites positioned within an average elliptical outline of the medullary neuropil. O22, O72, O1, and O9 are the assigned names of SFs having unique corneal receptive fields, after Wiersma and Yamaguchi, 1966.
FIGURE 6

CORRESPONDENCE OF SF DENDRITIC PROJECTIONS AND CORNEAL RECEPTIVE FIELDS

Sketches of the corneal outline, divided approximately into quadrants, are presented for each of five identified SFs. The corneal receptive fields used to identify the individual neurons are filled in black. Tracings of lucifer yellow fills of SFs with the given receptive fields are shown in the right column. An elliptical outline of an averaged medullary neuropil, divided into quadrants, is superimposed on the SF dendritic fields. The elliptical nature of the Medulla is exaggerated because the neuropil lies at 45° with respect to the viewing plane. The higher order SF dendrites are restricted to that portion of the Medulla corresponding to the appropriate corneal receptive field. Calibration bar, 100 μm. Abbreviations: D, dorsal; A, anterior; P, posterior; L, lateral; M, medial.
orientation occurs. All thirty-four SFs whose receptive fields were determined and dendritic fields examined conformed to this correlation.

When multiple SFs were filled in a single preparation, it was found that as expected, SFs with unique but overlapping receptive fields have portions of their dendritic trees which overlap and portions that are separate (Fig. 7A).

In summary to this point, each vertical sheet of SF dendrites is sandwiched in the Medulla and oriented perpendicular to the incoming retinotopic projection from the Lamina. Apparently the SF dendritic projection maps its receptive field through that portion of the retinotopic mosaic with which it intersects.

In order to more critically test the above hypothesis, contour sensitivity maps were obtained for 019 in four different preparations (Fig. 8). In Pacifastacus 019 is most sensitive near the central pole and just below the horizontal midline (triangles in Fig. 8). This is very similar to 019 in Procambarus as reported by Glantz (1973). However, 019 in Pacifastacus is very insensitive dorsal to the horizontal midline and in most cases its sensitivity extends into the anterior-ventral quadrant (Fig. 8). These results are from contour sensitivity maps obtained from fibers which were unequivocally identified as 019 with an intense hand-held light source. It appears that SF identifications based solely upon contour sensitivity maps using a dim point source would be less reliable than those determined with a small but
FIGURE 7
MULTIPLE SUSTAINING FIBER
DYE-FILLINGS

Tracings of dorsal (D) and medial (M) views of two
SFs injected with lucifer yellow in the same pre-
paration. The dashed lines indicate the outline of
the Medulla.
FIGURE 8

ISOSENSITIVITY CONTOUR MAPS OF O19 VISUAL RECEPTIVE FIELDS

Four different O19 preparations whose visual fields were mapped with a point light source. Each visual arc was tested twice in five degree intervals, the spike counts averaged and converted to Log I sensitivity (see Materials and Methods). The points from each arc having the same sensitivity (-2.0, -1.0, or -0.5) were connected to give isosensitivity contour lines. Triangles indicate the location of points of maximal sensitivity. Abbreviations: A, anterior; D, dorsal; P, posterior; V, ventral. 0° = central pole of the eye.
FIGURE 8
intense hand-held light source. What the contour sensitivity maps were useful for was a quantitative comparison of the change in sensitivity surrounding the most sensitive receptive region of O19s from different animals. This could then be correlated with changes in dendritic density in the corresponding regions of the neuropil. The dorsal-ventral visual arc passing through the central pole was chosen for the comparison. Without prior knowledge of the particular columns in the Medulla subserving a particular visual arc, the dorsal-ventral arc was compared with the dorsal-ventral midline of the medullary neuropil (Fig. 9). The intervals of length between successive O19 dendritic processes were converted to dendritic frequency (number per millimeter of medullary neuropil), and plotted as a function of the dorsal-ventral position in the Medulla (Fig. 10B). Although these measurements are not from the neurons used to obtain Figure 10A, there is present a positive correlation between the sensitivity to light and dendritic density along the dorsal-ventral arc of the eye (Fig. 10A and B).

B. DISCUSSION

The small number (14, Wiersma and Yamaguchi, 1966) of crayfish SFs, and the fact that, combined, they represent the entire visual field for each compound eye, make this a favorable preparation for studying the central representation of visual space in terms of the dendritic projections of identified neurons. The SFs are topographically
FIGURE 9

RECONSTRUCTIONS OF FOUR OL9 LUCIFER YELLOW FILLS

Tracings of OL9s from four different preparations. Each was centered within the Medulla and the dorsal-ventral midline superimposed on the dendritic branching pattern. The length intervals between successive branches along this line were measured, converted into dendrite frequency per millimeter and plotted in Figure 10B.
FIGURE 10
CORRELATION BETWEEN RELATIVE SENSITIVITY AND DENDRITIC DENSITY ALONG THE DORSAL-VENTRAL ARC OF THE EYE

A. Log I sensitivity along the dorsal-ventral arc of the eye, four different preparations taken from Figure 8. Each preparation was normalized so that Log 0 equaled its greatest absolute sensitivity value. 0° = central pole of the eye.

B. Dendritic frequency or Ë dendrites (number of dendrites/millimeter) as a function of the dorsal (D)-ventral (V) position along the midline of the Medulla. The dorsal edge of the neuropil equals 0, the ventral edge equals 1.0. The points are averages (+ standard deviation) taken from measurements of the four O19s in Figure 9. The dendritic frequencies were averaged over 0.2 relative D-V distances. Note that the peak dendritic frequency corresponds in D-V position to the peak Log I sensitivity along the dorsal-ventral visual arc in A.
FIGURE 10
organized within the Medulla according to their corneal receptive fields. I suggest that the mapping of the secondary and higher order dendritic branches is the major factor determining each SF visual receptive field. This hypothesis is supported by the fact that most synapses on arthropod neurons appear only on the higher order dendrites within the neuropil (Krasne and Stirling, 1972; King, 1976; Bishop and Bishop, 1981).

It is common for the most peripheral SF branches to extend slightly beyond their expected boundaries. In this context it should be noted that published contour sensitivity maps (Glantz, 1973; Woodcock and Goldsmith, 1973; present study) show that the excitatory receptive fields of many SFs are more extensive than those reported by Wiersma and Yamaguchi (1966).

The SFs represent an alternate functional pathway in parallel with the continuing retinotopic projection through the more proximal optic neuropils (Strausfeld and Nassel, 1981). The SFs' response properties and postsynaptic targets, when compared to those of visual interneurons originating within the Lobula, support this contention. Preliminary observations (n=2) suggest that some of the jittery movement detectors arise in the Lobula (see Section III, C). The motion detectors are the only cell type emerging from the eyestalk with the appropriate response properties to trigger a defense reflex (Glantz, 1974). These cells presumably utilize the retinotopic organization which reaches
the Lobula through the second optic chiasma.

The postsynaptic targets of the SF Lateral Protocerebral terminals are unknown. However, the X-organ cells originate in this optic lobe and the SFs may be part of the neural pathway for light-adaptation hormone release from the sinus gland (Barrera-Mera and Berdeja-Garcia, 1979). Alternatively, these SF terminals may synapse with higher order visual interneurons or oculomotor neurons (Mellon and Lorton, 1977).

The choice of the dorsal-ventral arc to compare visual sensitivity and dendritic density was not entirely arbitrary. This visual arc of O19 contains the largest variation in sensitivity along its length (nearly 2.5 log units). It also can be imposed on the medullary neuropil with the least ambiguity (the dorsoventral midline). The fact that dendritic density is positively correlated with visual sensitivity supports the contention that the visual receptive field is mapped by the SF dendrites (Kirk et al., 1982). The more dendrites per unit area results in more retinotopic columns contacted, and therefore, a stronger contribution from the corresponding area of visual space. Motion detectors in the fly have receptive fields governed by the same principle (Eckert and Bishop, 1978).

Other factors which could contribute to the contour of SF visual sensitivity are: 1) electrotonic distance of synapses from the spike initiating zone, 2) varying density of synapses along the dendrites, 3) unequally weighted synaptic inhibition, and 4) gradients of synaptic strength.
1) Variation in electrotonic distance

The SF dendrites electrotonically (passively) propagate synaptic activity to the spike initiating zone (see Sect. II, C). It is, therefore, possible that anatomically remote synapses, impinging on dendrites furthest from the spike initiating zone, would suffer heavily from electrotonic decrement. However, the O19 dendrites, corresponding to the most sensitive part of its visual field, are anatomically among those most distant from the spike initiating zone. Also, compartmental electrotonic model (Rall, 1959) assuming a uniform specific membrane resistivity suggests a maximum decrement of a DC electrotonic potential by fifty percent (Brian Waldrop, personal communication), whereas the light sensitivity along the dorsal-ventral arc may change by 2.5 log units.

2) Variation in synaptic density

Another possibility is a systematic and continuous change in the density of excitatory synaptic innervation. This alternative awaits confirmation by electron microscopy. A similar type of phenomenon has been documented in motion detectors of the fly (Eckert, 1981).

3) Variation in weighting of synaptic inhibition

A form of synaptic inhibition exists throughout the SF visual receptive field (Glantz, 1973; Wiersma and Yamaguchi, 1967; see Sect. II, C). The possibility exists that synaptic inhibition increases in its effectiveness farther away
from the "hot spot" of a SF's receptive field. This could come about by, a) strategic location of inhibitory synapses, b) variation in inhibitory synaptic density, and/or c) changes in inhibitory synaptic strength. Again, electron microscopy of synaptic profiles may clarify this situation.

4) Variation in synaptic strength

Finally, excitatory synaptic strength may depend on the position within a SF dendritic tree. This would require that the dendrites possess the ability to induce a characteristic synaptic strength of synapses impinging on them. Quantal analysis of synapses at various levels within a SF's dendritic field and/or electron microscopy of presynaptic densities (Govind and Chiang, 1979) would help verify this hypothesis.
II. SUSTAINING FIBER PHYSIOLOGY

A. INTRODUCTION

Once it became possible to obtain intracellular recordings from SF integrative zones a number of questions regarding the physiology of SF visual responses were directly testable. Following is a list of questions which will be addressed in the results:

1) What is the nature of the SF synaptic response to light?
2) Is there any spontaneous synaptic activity in the dark?
3) Do SF membrane properties account for a part of their unique reaction to light (e.g., response waveform, adaptation, bursting, pacing, post-excitation depression)?
4) Do SFs interact synaptically to produce their observed correlations and/or lateral inhibition (Glantz and Nudelman, 1976)?

B. MATERIALS AND METHODS

The crayfish were prepared for intracellular recording as described above (Sect. I, B). The microelectrodes were filled with lucifer yellow when recording only or with 3M KCl where recording and current passing ability was needed. After beveling, the KCl electrodes had D.C. resistances from 20 to 50 megaohms.

When determining I-V and I-f relationships the Dagan 8100 Single Electrode System electrometer was used. The
Dagan 8100 possesses a switch current clamp mode which alternates cycles of current injection with cycles of voltage recording and thus allows membrane potential measurements without distortion due to resistance changes by the recording microelectrode and/or the IR drop across the microelectrode (Muller, 1973). Current-voltage data were obtained from the steady state response, and current-frequency plots were made by doubling the spike count during the first 500 msec following current onset.

C. RESULTS

The observations reported in this section are taken from 88 SF integrating segment (IS) impalements. The IS of a SF includes the major transverse process within the Medulla as well as the tapering portion of the SF proximal to the Medulla, leading to the spike initiating zone. Intracellular impalements of this region revealed large resting membrane potentials (mean = (-)67.8 mV), passively conducted action potentials (mean amplitude = 14.9 mV) and large visually induced excitatory postsynaptic potentials (EPSPs, Fig. 11, Table II). In the dark, SFs exhibit spontaneous EPSPs (Fig. 11A). Even in this dark condition the synaptic potentials appear to be compound events with a great deal of summation taking place. No discrete unitary EPSPs could be resolved.

The response to light-on is illustrated in Figure 11B. As discussed in detail above, SFs respond to a step increase in illumination with a transient peak and a steady state
FIGURE 11
INTRACELLULAR RECORDINGS
FROM SUSTAINING FIBERS

A. Spontaneous PSP activity in the dark (019) recording. Abbreviations: int, intracellular SF recording; ext, extracellular recording from SF bundle in optic tract. Calibrations: 10 mV, 200 msec.

B. On- and off-responses to a light pulse (indicated in bottom trace) delivered via a fiber optic light guide to a region overlapping the excitatory receptive field and inhibitory surround. Calibrations: 10 mV, 200 msec.

C. Multiple traces superimposed illustrating the one for one correlation between intracellular action potential and extracellular spike. Each oscilloscope sweep was triggered on the rising phase of the intracellular action potential. Calibrations: 10 mV, 2 msec.

D. Antidromic action potentials. The extracellular optic tract electrode was switched to a pulse generator and suprathreshold stimuli (5V, 0.5 msec) were delivered to the axon of the impaled cell. Calibrations: 10 mV, 5 msec.
<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>$\bar{X}$</th>
<th>±</th>
<th></th>
</tr>
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<tr>
<td>Resting Membrane Potential</td>
<td>22</td>
<td>67.8 ± 8.0 mV</td>
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<td>Spike Height</td>
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<td>Intracellular to Extracellular Delay</td>
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<td>2.0 ± 0.8 msec</td>
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<td>Input Resistance</td>
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<td>Time Constant</td>
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<td>Transient On-Response</td>
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<td>Steady State Response</td>
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<td>Off-Response:</td>
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<td>Delay</td>
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<tr>
<td>Magnitude</td>
<td>22</td>
<td>9.1 ± 5.4 mV</td>
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plateau. If a significant amount of light falls outside of the excitatory receptive field, an off-response occurs at a delay (Fig. 11B, TABLE II). The on- and off-responses appear to be massive, light induced summations of EPSPs. These EPSPs result from an increase in conductance presumably to sodium ions (or in combination with other ions, the estimated reversal potential = (-)19 mV). Changes in membrane input conductance during light responses are often small in size. Apparently the modest input resistance of the IS (mean = 7.8 megohms, Fig. 13, TABLE II) and the length of inactive membrane between the recording site and site of synaptic input are sufficient to substantially isolate the recording microelectrode from the synaptic conductance changes. The same principle may also result in a substantial amount of isolation of the primary dendrites from each other, therefore leading to a linear summation of their synaptic input (Arechiga and Yanagisawa, 1973; Yamaguchi and Otsuka, 1973).

During the SF transient response the instantaneous spike frequency can exceed 250 impulses per second (Glantz, 1971). The sustained response (i.e., during continued illumination) has a much lower spike frequency that is between one and ten impulses per second (Wiersma and Yamaguchi, 1966). As might be expected this dramatic adaptation in spike frequency results from a large decrease in the summated amplitude of the light induced EPSPs (personal observation). This correlates nicely with the linear current-voltage relationship of the IS (Fig. 13) and the linear current-frequency relationship.
FIGURE 12
SUSTAINING FIBER RESPONSE
TO EXTRINSIC DEPOLARIZATION
AND INTENSE LIGHT STIMULI

A. The impaled SF was depolarized with a pulse of extrinsic current (monitored in bottom trace). Calibrations: 10 mV, 5 nA, 100 msec.

B. Periodic bursting in a SF during continuous intense but diffuse illumination. Note the correlated activity in other SFs monitored in the extracellular lead. Calibrations: 10 mV, 100 msec.

C. Pacing activity in the SF illustrated in B following a period of light adaptation. Note the periodic compound EPSPs occurring at a slightly longer time period than in B. Again there is correlated activity in other SFs (bottom trace). Calibrations: 10 mV, 100 msec.

D. Postexcitation depression. An intense light pulse was delivered to the excitatory receptive field of the impaled SF (O19). Following the light-on response the membrane hyperpolarizes and slowly recovers over a period of seconds during which time spontaneous activity reappears due to a dim room light. Calibrations: 10 mV, 1 sec.
FIGURE 13
SUSTAINING FIBER CURRENT-VOLTAGE RELATIONSHIP

The steady state response to extrinsic currents are plotted for a typical SF preparation. The line is drawn by eye, $R_{\text{input}} = 4.7$ megaohms. Examples of a SF response to hyperpolarizing and depolarizing currents is shown in the inset.
(typical of SFs, Fig. 14A) suggesting that reduced synaptic currents are linearly translated into a decreased spike frequency. This requires that the instantaneous spike frequency be linearly related to the amplitude of the underlying EPSPs at all times during the light response. Per contra, the slope of this relationship changes significantly within the first 500 msec of a light response (Fig. 14B). However, as can be seen in Figure 12A, a comparable amount of adaptation occurs in the spiking response to extrinsic current, and therefore, it appears that the decrease in spike frequency during a light response can be entirely explained by EPSP diminution and action potential frequency adaptation (Fuortes, 1959).

In 24 of the SF impalements an intense flash of light gave rise to a subsequent prolonged hyperpolarization (Fig. 12D). This hyperpolarization consisted of a dramatic suppression of ongoing EPSPs which regain their prominent summation following a prolonged (seconds) recovery period. This postexcitation depression was observed by Wiersma and Yamaguchi (1967) when they obtained an inhibitory effect of comparable time course with two light spots in a SF's excitatory receptive field. Sustaining fiber spiking in response to extrinsic currents is also suppressed during this time period following a light response (personal observations). However, the effect cannot be mimicked by strong driving with extrinsic depolarization. I conclude that postexcitation depression in SFs is a postsynaptic inhibition elicited by
FIGURE 14

SUSTAINING FIBER CURRENT-FREQUENCY AND VOLTAGE-FREQUENCY RELATIONSHIPS

A. Current-frequency relationship for a typical SF impalement. The number of action potentials occurring during the first 500 msec of a current step was doubled to give impulses per second ($\bar{f}$ (Hz)). The points are averages from at least two current steps at each current level. The line was drawn by eye.

B. Voltage-frequency relationship. The interspike interval between two spikes at the peak of the transient and the last two spikes at the end of the steady state response were measured, along with the envelope of the underlying membrane potential. These measurements were made from a number of light responses (stimulus duration = 500 msec) taken from a response-intensity series ($V_m$/Log I). The interspike intervals were converted into impulses per second ($\bar{f}$ (Hz)) and plotted as a function of their underlying membrane potential ($V_m$(mV)).
FIGURE 14
light-on within the excitatory receptive field. It should be noted that the existence of inhibition within SFs' receptive fields had been proposed by Glantz (1973) and Wiersma and Yamaguchi (1967).

Finally, the mechanism responsible for SF bursting during intense and diffuse illumination was investigated. As described previously (Glantz and Nudelman, 1976) SFs produce a steady pacing and/or bursting discharge when large parts of the cornea are placed in bright light (e.g., five milliwatts per square centimeter). Under the same conditions several SFs exhibit strong crosscorrelations indicative of either excitatory interconnections or common presynaptic input. Figure 12B and 12C document that: 1) bursting arises from periodic compound EPSPs produced in SFs by oscillating presynaptic elements, and 2) the coincidence of SFs' preferred times of firings results from shared (common) presynaptic input. No SF-SF interactions which might contribute to the synchronous bursting were observed, i.e., no EPSPs were found to be correlated with neighboring SF spikes in the extracellular lead, and neighboring SFs were not activated when driving the impaled SF with extrinsic current. Sustaining fibers are not endogenous bursters as their spiking response to injected current is an unpatterned rhythmic discharge (Fig. 12A). It is, therefore, likely that the oscillatory synaptic input is an emergent property of a network of neurons presynaptic to the SFs (i.e., within the Lamina or outer Medulla) (Nudelman and Glantz, 1977).
D. DISCUSSION

Although it could be argued that the SF synaptic dark activity results from the effects of stray light, my impression is that spontaneous EPSPs are normally present in the dark and that the effect of light is to increase their frequency of occurrence. Even at low light intensities, the EPSPs summate to the extent that individual events are not discernible. The outcome is a noisy plateau which with increasing steps of illumination exceeds the threshold for action potential production.

Sustaining fibers are remarkable in their lack of interactions amongst themselves. Preliminary evidence also suggests that they do not make synaptic connections with other visual cells originating within the Medulla or Lobula (e.g., dimming units, phasic-on-off cells, motion detectors). The SFs do exhibit lateral inhibition (Wiersma and Yamaguchi, 1967; Arechiga and Yanagisawa, 1973; Yamaguchi and Ohtsuka, 1973). However, again it does not result from SF-SF interactions or SF interactions with any other descending visual cells originating within the second layer of the Medulla (personal observations). The SFs do exhibit postsynaptic inhibition resulting from light spots onto their inhibitory surround (Waldrop and Kirk, in preparation). A more detailed evaluation of SF synaptic relationships awaits electron microscopy of their dendrites.

In a superficial way SF postexcitation depression resembles the self-inhibition of Limulus eccentric cells
(Purple and Dodge, 1966). It builds up and decays with a long time constant, making a contribution to light adapta-
tion (Laughlin, 1981), and it is a form of inhibition within the cell's excitatory receptive field. The major difference being that an eccentric cell feeds back onto itself to pro-
duce the inhibition whereas a SF does not. Apparently SF postexcitation depression arises from synaptic inhibition produced by presynaptic cells within the retinotopic columnar organization.

Periodic bursting activity has been observed in many visual systems (for a review see Glantz and Nudelman, 1976). In motor systems it is often associated with endogenous mem-
brane properties (Stent et al., 1976) or periodic bouts of synaptic inhibition (Selverston et al., 1976). In the Lim-
ulus lateral eye, eccentric cells which participate in a lateral inhibitory network exhibit periodic bursting (Kaplan and Barlow, 1975), and theoretically it has been shown to be an emergent property of the inhibitory network (Coleman and Renninger, 1975). Nudelman and Glantz (1977) forwarded a similar hypothesis for the SFs. In their model, the lateral inhibitory interactions occurred among a network of neurons presynaptic to the SFs. This has been confirmed by my intra-
cellular recordings of SF bursting activity, and it should be noted that, although a contribution of postsynaptic SF inhibition cannot be entirely ruled out, inhibition onto SFs must play a minor role in SF burst production.
III. OTHER VISUAL CELL TYPES IN THE EYESTALK

A. INTRODUCTION

It was important to know what other visual cell types are present and how they are morphologically related to the optic ganglia. This would indicate the complexity of visual processing that is present and the location of various functional operators in the neuropil sequence (i.e., Lamina, Medulla, etc.). This section describes the remainder of visual interneurons encountered in this study. A group of neurosecretory cells which also appear to be visual interneurons are discussed in the subsequent section.

B. MATERIALS AND METHODS

The semi-intact eye-cup was used as described in Section I, B. The cells to be presented were penetrated at the proximal edge of the Medulla except for three examples of Lamina impalements. In order to record from the Lamina the dissection was extended to the edge of the cornea and the Lamina was desheathed on its dorsal aspect. Lucifer yellow electrodes were used for recording and subsequent dye injection.

C. RESULTS

The visual interneurons most frequently encountered possessed axons within or nearby the tract of SPISs proximal to the Medulla. Cells impaled in this area were generally either tonically excited or inhibited by step increases in illumination. Figure 15 presents the reconstructed
FIGURE 15

PHASIC LIGHT-ON CELL

Tracing of a lucifer yellow fill of a visual interneuron that responded to light-on (inset). Arrowheads indicate times of light-on and -off. Abbreviations: M, Medulla; L, Lobula; LP, Lateral Proto-cerebrum.
morphology of a rapidly adapting (phasic) light-on interneuron. The inset documents the effect of light-on and -off. This visual interneuron has a uniplanar dendritic tree much like the SFs, however, it is confined entirely to the Serpentine Layer. The axonal process terminates in the Lateral Protocerebrum (LP) where it branches sparsely.

Dimming fibers are also commonly penetrated near the Medulla. Figure 16 is an example of a tonic light-off unit which possesses a uniplanar sheet of dendrites within the Serpentine Layer and projects to the LP. The insets demonstrate the inhibitory effect of light-on (A) which reverses with one nanoamp of hyperpolarizing current (B). The dimming fibers most commonly impaled were anatomically restricted to the optic ganglia (see Discussion).

On occasion the site of impalement was sufficiently proximal so that motion detectors were penetrated (Fig. 17). These neurons originate in the Lobula (L) and send their axons into the optic tract. The inset illustrates the response to passing a dark bar between the microscope lamp and the cornea. As is typical for jittery motion detectors (Wiersma and Yamaguchi, 1966) they responded to light-off and their response rapidly habituated. Other intracellular recordings revealed large motion-induced EPSPs subthreshold for spike initiation (personal observations), again characteristic of a rapidly habituating response.

While attempting neuropilar penetrations inside the Medulla, I encountered non-spiking interneurons (NSI) that
FIGURE 16

TONIC LIGHT-OFF CELL

Tracing of a visual interneuron that was inhibited by light-on (inset). A-inset documents the response of the cell to light-on and -off (arrowheads). B-inset illustrates the reversal of the light induced IPSP by one nanoamp of hyperpolarizing current (on continuously during the trace).
FIGURE 17

OPTIC TRACT MOTION DETECTOR

Tracing of a visual interneuron that responded to light-off and jittery motion (inset). The line underneath the inset trace indicates the approximate time that a dark bar was passed between the microscope light and the eye.
responded to pulses of light (Fig. 18 inset, A and B). Figure 18 contains tracings of medial (M) and dorsal (D) views of a NSI filled with lucifer yellow. Although the processes were extensive and difficult to follow, it was apparent that the impaled cell was dye-coupled (Glantz and Kirk, 1981) to a morphologically similar cell, presumably another NSI. The NSI possessed a bilanar dendritic arrangement paralleling that of the SFs (Fig. 18, D).

A peculiar cell type often impaled along the same electrode tract as the SFs is presented in Figure 19. This cell was tonically active and responded phasically to light-on (shown in Fig. 19 inset) and -off. Cells of this type often also respond to tactile stimulation of the thoracic and abdominal carapace. The dendritic (?) projection within the Medulla is extensive (Fig. 19), bilanar and enters the second medullary tangential cell layer as well as the Serpentine Layer (not shown). These cells are peculiar in that they have no cell body in the eyestalk and their action potentials appear to arise from the baseline. They are excited by ipsilateral visual stimuli only and the visual responses are produced by summatting EPSPs (Fig. 19 inset). It appears that these interneurons may be bidirectionally conducting interneurons with separate spike initiating zones subserving the tactile and visual inputs.

As a preliminary to a study of the transmission of visual signals between the Lamina Ganglionaris (LG) and Medulla (M), a survey of intercellular response and morphological
FIGURE 18

LOCAL NONSPIKING INTERNEURONS

Tracings of medial (M) and dorsal (D) views of two local interneurons located in the Medulla. A single cell was impaled and injected with lucifer yellow. Both cells with nearly identical morphologies were filled from the single injection. A-inset is an example of the light response of the cell that was injected. Light-on is at the beginning of the trace, light-off is indicated by the arrowhead. B-inset is another example of a recording from a nonspiking interneuron, recorded in the Medulla, which exhibited an exaggerated transient response to light-on (when compared to its steady state response). Sustaining fiber responses are included for comparison (extracellular recordings, bottom traces).
FIGURE 19
PHASIC LIGHT-ON AND -OFF CELL

Tracing of a neuron which responded phasically to light-on and -off. Dotted light is the outline of the medullary neuropil. The inset illustrates the response of the cell to light-on (arrowhead) and continuous light (to the end of the trace). Note the presence of spontaneous dark activity before light-on. Calibrations: 10 mV, 500 msec.
cell types was performed. Graded hyperpolarizing (Fig. 20B) and depolarizing (with action potentials, Fig. 20E) responses could be attributed to monopolar morphological cell types (Fig. 20A). Another morphological cell type, a LG tangential interneuron (Fig. 20C) was also associated with graded hyperpolarizing responses to light-on (Fig. 20D). This neuron possessed an unusually large axonal process within the first optic chiasma which enabled a long and stable intracellular recording. The on- and off-responses were both due to increases in conductances with reversal potentials negative with respect to resting membrane potential (personal observations).

D. DISCUSSION

During this study optic tract dimming units were encountered infrequently. Preliminary observations (n=1) indicate that they originate within the Medulla as do the SFs. It was not established, however, in which medullary layer their dendrites are located, only that they possess transverse processes at the proximal edge of the Medulla. Another cell type with a similar morphology was filled twice. Its response properties resembled those of medium movement fibers found in the crab and rock lobster (Wiersma et al., 1982). Perhaps its extremely small axon diameter (less than five micrometers) explains why it was previously not encountered in the optic tract of the crayfish (Wiersma and Yamaguchi, 1965).
FIGURE 20

EXAMPLES OF CRAYFISH
Lamina Interneurons
AND THEIR LIGHT RESPONSES

A. Reconstruction of a lucifer yellow fill of a
Lamina Monopolar Cell (LMC) positioned within dot-
ted outlines of the neuropils (LG, Lamina Ganglion-
micrometers.

B. Example of a graded, light-on hyperpolarization
recorded from a LMC. Included is an extracellular
monitor of the SF response (bottom trace). Calibrations: 5 mV, 100 msec.

C. Reconstruction of a lucifer yellow fill of a Lamina
tangential neuron, medial view. Abbreviations: M,
Medulla; LG, Lamina Ganglionaris. Calibration:
200 micrometers.

D. Intracellular response of the tangential cell in C
to light-on and -off. Calibrations: 5 mV, 200 msec.

E. A depolarizing, spiking response recorded in a LMC.
The SFs were monitored extracellularly (bottom
trace). Calibrations: 5 mV, 100 msec.
The nonspiking amacrine cells in Figure 18 have not been observed previously in Golgi studies (Strausfeld and Nassel, 1981). The similarity of their response to light, their broad receptive fields and anatomical proximity to the SFs suggest a role in the production of lateral inhibition found in SFs (Waldrop and Kirk, unpublished observations). The other cell type which satisfies the above criteria for participation in lateral inhibition is the Lamina tangential neuron illustrated in Figure 20C. This neuron appears identical in its morphology to the TAN2 neuron described by Nassel (1977). It possesses a vertical process proximal to the Lamina neuropil which extends along the entire extent of this ganglion. Although they were not resolved microscopically, fine branches presumably contribute to the optic cartridges along the length of this vertical process. The medullary (axonal?) terminals penetrated to the second tangential cell layer of the Medulla, at the level of the SFs. The Lamina monopolar cells (Fig. 20A) also terminated at this level.
IV. THE CRAYFISH MEDULLA EXTERNA X-ORGAN

A. INTRODUCTION

The decapod crustacean eyestalk contains important neurosecretory structures (Hansson, 1948). These include the Medulla Terminalis X-Organ (MTXO) and sinus gland (SG), the axonal endings of the MTXO cells which terminate in a neurohemal space. Numerous neurohormones affecting many organ systems and behaviors of the animal are thought to be released from the SG (for a recent review see Andrew, 1982). Another, and less conspicuous X-Organ, also thought to contribute axons to the SG, has been documented in Natantians, (shrimps, Bellon-Humbert et al., 1981), Brachyurans (the true crabs, Bliss and Welch, 1952; Matsumoto, 1958) and Anomurans (the hermit crabs and crab-like forms, Bursey, 1975). These cell bodies are clustered over the Medulla Externa X-Organ (MEXO). This structure has not been previously described in a Macruran (crayfish and lobster) where only neurosecretory cells scattered over the Medulla Externa have been reported (Andrew et al., 1978; Durand, 1956; Shivers, 1967).

A characteristic feature of crustacean neurosecretory cells is a blue-white appearance in fresh tissue. When treated with paraldehyde fuchsin (Gormori, 1950) they generally stain positive, although the stain is often restricted to the neurohemal sites of release (Andrew, 1982; Andrew and Saleuddin, 1979). The axon terminals of neurosecretory cells in invertebrates and vertebrates contain dense core
vesicles typically 140-250 nanometers in diameter (Mason and Bern, 1977).

Crustacean neurosecretory cells have electrophysiologi-
cal characteristics which distinguish them from non-neuro-
secretory neurons. Here neurosecretory implies that a neuro-
hormone is released into the blood which transports it to a
distant effector organ. This is in contrast to a fast act-
ing neurotransmitter released at a nearby specialized syn-
aptic site. Iwasaki and Satow (1971) made intracellular re-
cordings from MTXO cell bodies in the crayfish. Action po-
tentials actively invade these somata and have complete dur-
ations of about 8-12 msec. In fact, it appears that action
potential duration as recorded in the cell body is a feature
that distinguishes neurosecretory from other neuronal cell
types, neurosecretory cells universally having action poten-
tials of long duration (Andrew, 1982; Mason and Bern, 1977).
Synaptic potentials have also been recorded in crustacean
neurosecretory cells (Iwasaki and Satow, 1971; Konishi and
Kravitz, 1978), however, these synaptic potentials were in
response to nerve shock and the normal mechanisms for acti-
vating these pathways are unknown.

In this section I describe initial observations on the
MEXO in the crayfish. Included will be a combination of
light microscopy, intracellular dye-filling, and documenta-
tion of a modality specific visual synaptic response.
B. MATERIALS AND METHODS

The cell bodies of the neurosecretory cells were visually impaled. If the cellular morphology was desired the electrodes contained lucifer yellow for intracellular iontophoresis. Alternatively the microelectrodes contained 3M KCl for current passing while recording. Again the Dagan 8100 Single Electrode System was used in order to obtain accurate steady state membrane potential measurements.

The paraaldehyde fuchsin stain was performed according to Gomori (1950) on ten micrometer paraffin sections. The counterstain was a mixture of Fast Green, Chromotrope and Orange G1.

C. RESULTS

During the course of the experiments described in the preceding sections, a discrete cluster of cell bodies with a blue-white appearance in reflected light was discovered. These somata are among the largest neuronal cell bodies present in the crayfish eyestalk, averaging 34.3 micrometers in diameter (TABLE III, as determined from lucifer yellow injections). Generally the cells are tightly clustered at the dorsomedial edge of the Medulla (or Medulla Externa), therefore, by analogy with similar structures in other decapods I shall refer to them as the Medulla Externa X-Organ (MEXO).

Their cellular morphology as revealed by lucifer yellow injections is summarized in Figures 21 and 22. The cell body gives rise to a small neurite that abruptly branches
FIGURE 21

WHOLEMOUNT PREPARATIONS OF LUCIFER YELLOW FILLS OF MEXO NEUROSECRETORY CELLS

A. Lateral view of a neurosecretory cell illustrating its planar dendrites in the Medulla (M) and its two axons going out of focus as they approach the Lamina Ganglionaris (LG). Dorsal is to the right. Scale = 200 micrometers.

B. Same preparation as in A; with a focus on the Lamina terminals. Abbreviations: M, Medulla; LG, Lamina Ganglionaris. Scale = 200 micrometers.

C. Another lucifer yellow fill of a neurosecretory cell. In this preparation numerous other (neurosecretory?) cell bodies were dye-coupled to the injected cell -- with the largest (and brightest) cell body. Only the impaled cell exhibited any dendrites in the medullary neuropil (M). Dorsolateral view. Scale = 200 micrometers.

D. The same preparation as in C, dorsal view, low power illustrating the axon coursing dorsally into the Lamina Ganglionaris. Abbreviations: LG, Lamina Ganglionaris; M, Medulla; L, Lobula; LP, Lateral Protocerebrum. Scale = 500 micrometers.
<table>
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<tr>
<td>Primary Dendrites</td>
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<td>Tertiary Dendrites</td>
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<tr>
<td>Axon Terminals</td>
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<td>4.1 ± 2.8</td>
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</table>

Mean diameters in micrometers ± S.D.)
FIGURE 22

RECONSTRUCTION OF A MEXO NEUROSECRETORY CELL

Lateral view of MEXO neurosecretory cell illustrating its planar dendrites within the Medulla (M) and its multiple axon terminals in the Lamina Ganglionaris (LG). This is a composite tracing made from photographs of the lucifer yellow fill in Figure 21A & B.
into a profusion of fine dendrites within the Medulla and one or two axons which travel in a tract to the Lamina Ganglionaris. The dendrites lie in a single vertical plane within the second tangential cell layer of medullary neuropil. They cover an extensive vertical portion of the Medulla which is correlated with their large corneal receptive fields (see below). Although the axons do not terminate in a discrete neurohemal space such as the sinus gland they do expand proximal to the Lamina Ganglionaris (LG) and appear to send fine branches into a number of Lamina optic cartridges (Fig. 21B). Preliminary results (n=2) using HRP intracellular injections suggest an impressive expansion underneath the LG with numerous terminals contributing to the laminar array in a supraperiodic fashion.

The affinity of neurons for paraldehyde fuchsin (PAF), once thought to be universally indicative of a neurosecretory function (but more recently found not to be the case, Andrew, 1982, and personal communication) was tested on paraffin sections of four eyestalks. Figure 23 illustrates the typical results. The sinus gland (SG) and some regions of ganglionic sheath accumulated PAF, turning a deep purple hue (appearing dark in the black and white photograph of Figure 23). Only very light, violet, nonspecific staining appeared elsewhere in the eyestalk. The cell bodies of the MEXO, although with cytoplasm appearing quite granular at the light microscopic level (Fig. 23 arrowhead), did not stain with PAF. However, in the same eyestalk, the MTXO also did not stain
FIGURE 23
PARALDEHYDE FUCHSIN
(PAF) STAIN OF THE DISTAL EYESTALK

Horizontal section (10 micrometers thick) taken in the region of the Medulla Externa X-Organ (MEXO). The PAF stained the sinus gland (SG) heavily but not the cell body of a MEXO neurosecretory cell (arrowhead). Only a very light nonspecific staining is present in the Medulla (M) and Lamina Ganglionaris (LG). The other neuronal (and nonneuronal) cell nuclei in this section are counterstained primarily by fast green and appear dark. Scale = 200 micrometers.
with PAF as is common for these organs in crayfish that are reared in the wild (Andrew and Saleuddin, 1979).

The electrophysiology of MEXO cell bodies corresponds to that found universally for neurosecretory cells (Fig. 24). Action potentials are extremely long in duration (mean total width lasting 26.5 msec). During the daytime (8:00 AM to 6:00 PM) in the dark, the cells appear to be normally silent with spontaneous action potential activity occurring infrequently (unless the cell was damaged during impalement). Only in prolonged darkness was it ever possible to record spiking activity (Fig. 24B). When spontaneous action potentials did occur or spikes were produced with extrinsic depolarization (Fig. 24C) successive action potentials occurring close together were decreased in height. It was also common to see action potential duration increase by as much as fifty percent. Spike broadening is a characteristic of molluscan somata (Strumwasser, 1967) and may be associated with a prolonged inward calcium current (Eckert and Lux, 1976); Merickel and Gray, 1980). The MTXO spikes were shown to be dependent on both sodium and calcium (Iwasaki and Satow, 1971). The axon (or dendritic?) action potentials were shorter in duration (one-half width of less than two milliseconds) and did not display a spike height decrease or broadening with relatively high frequency driving (Fig. 24D). Presumably, therefore, the axon action potentials are solely sodium dependent as in molluscan neurons (Kandel, 1976).

The input resistance of MEXO cells is unusually large,
A. Action potential duration. Two action potentials superimposed illustrating their long duration. Cell body recording. Calibrations: 10 mV, 5 msec.

B. An example of spontaneous neurosecretory cell activity recorded in the soma with the preparation in continuous darkness. Calibrations: 10 mV, 500 msec.

C. Response of a cell body to extrinsic depolarizing current. Note the decrease in spike height and slight increase in spike width with successive firings. Calibrations: 10 mV, 1 nA, 100 msec.

D. Response of an axon to 1 nA extrinsic depolarizing current. Note the constant spike height, short spike duration and relative lack of action potential adaptation. Calibrations: 20 mV, 500 msec.
as was also reported for the MTOX somata (Iwasaki and Satow, 1971). The average input resistance of eight cells was 27.5 megaohms (TABLE IV), however, it could approach 50 megaohms (Fig. 25). This can be compared to input resistances obtained for crayfish abdominal motoneuron somata of similar size (5-21 megaohms, Kirk and Glantz, 1981b; Takeda and Kennedy, 1964). A small amount of delayed rectification was observed in the cell bodies (Fig. 25) and occasionally hyperpolarizing afterpotentials, following the spike after-hyperpolarization, leading into the pacemaker potential were seen. This could be indicative of an early potassium current as is found in molluscan somata (Connor, 1975). Along with large input resistances the MEXO somata exhibit long time constants. The mean value taken from nine preparations (and measured assuming a single time constant) was 143.9 msec (TABLE IV). The maximum time constant observed was 300 msec. These impressive values arise (in combination with a high specific membrane resistivity, \( R_m = 50,000 \) ohms-cm\(^2\)) from a large specific membrane capacitance, estimated to be five microfarads per square centimeter. The estimated value for specific membrane resistivity was obtained in collaboration with Brian Waldrop using a compartmental electrotonic model developed by Rall (1959). A computer program that iteratively arrives at a best \( R_m \) value which, when applied through the electrotonic model to the neuron's branching pattern and dimensions, results in a close approximation of the observed input resistance.
TABLE IV
SUMMARY OF NEUROSECRETORY CELL PHYSIOLOGY
(Means ± S.D.)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting Membrane Potential</td>
<td>6</td>
<td>68.0</td>
<td>4.6 mV</td>
</tr>
<tr>
<td>Spike Height</td>
<td>19</td>
<td>56.7</td>
<td>15.5 mV</td>
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<tr>
<td>Spike 1/2-Width</td>
<td>17</td>
<td>7.4</td>
<td>1.9 msec</td>
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<tr>
<td>Spike Total Width</td>
<td>17</td>
<td>26.5</td>
<td>5.9 msec</td>
</tr>
<tr>
<td>Input Resistance</td>
<td>8</td>
<td>27.5</td>
<td>13.1 MΩ</td>
</tr>
<tr>
<td>Time Constant</td>
<td>9</td>
<td>143.9</td>
<td>77.0 msec</td>
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<tr>
<td>IPSP (at rest)</td>
<td>16</td>
<td>(-)8.2</td>
<td>3.9 mV</td>
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FIGURE 25
NEUROSECRETORY CELL BODY
CURRENT-VOLTAGE RELATIONSHIP

The steady state responses in darkness, of two soma recordings, to extrinsic current steps are plotted as a function of the applied current. The data points are averages of at least two measurements at each current level. The line represents a least-squares linear regression fit with \( r = (\cdot)^{0.99} \) and the slope equals 47.5 megaohms.
The most remarkable characteristic of the MEXO cells is their response to light stimuli directed to the cornea (Fig. 26). These neurosecretory cells are tonically inhibited by light-on. The response is mediated by the retinal photoreceptors because light stimuli directed to other parts of the eyestalk (and the rest of the animal) are not effective in producing the response. However, the cells have extensive visual fields as light directed to any part of the cornea is able to produce an inhibitory postsynaptic potential (IPSP) in a MEXO cell.

Figure 26 illustrates the IPSP recorded in a MEXO soma in response to a light pulse (bottom trace). The response to light-on can be biphasic (Fig. 26A & C), but all portions of the PSP inhibit spiking (Fig. 26B). In addition all portions of the response become hyperpolarizing with sufficient depolarizing current. The IPSP is generally composed of an on-transient, a pause and a sustained response which lasts for the duration of the light pulse. Light-off responses are also commonly observed (Fig. 26C). These are followed by an extremely slow decay back to resting membrane potential. The response waveform, although exaggerated in delay and time course, notably resembles that of the sustaining fibers.

All components of the IPSP were affected by chloride ion injection through the recording electrode (Fig. 26C & D), therefore it is likely that the majority of the response is chloride mediated (Eccles, 1964). Iontophoresis of gamma-aminobutyric acid (GABA) mimics the light effect, shunts
FIGURE 26

A VISUALLY INDUCED SYNAPTIC POTENTIAL IN A NEUROSECRETORY CELL

A. Response of a MEXO neurosecretory cell to a pulse of light applied to the cornea (light monitor bottom trace). The SF response to the light pulse (second trace, extracellular recording) is presented for comparison of reaction times. Calibrations: 10 mV, 200 msec.

B. Example of the dependence of the light induced IPSP on membrane potential. Note also the ability of the IPSP to inhibit action potential production. Calibrations: 20 mV, 2 nA, 500 msec.

C. Example of another neurosecretory cell body recording with light mediated IPSP. Note on- and off-responses. Before chloride injection. Calibrations: 5 mV, 500 msec.

D. Reversal of the steady state IPSP and off-response following ten minutes of five nanoamps hyperpolarizing current. The recording electrode contained 3M KCl and the effect was presumably mediated by Cl injection. Calibrations: 5 mV, 500 msec.
and/or desensitizes the light response and produces an IPSP with the same reversal potential as the light induced IPSP, therefore, the IPSP produced by light-on is probably GABA mediated. Iwasaki and Satow (1971) observed IPSPs in response to electrical shock of the optic tract which were blocked by picrotoxin and presumably are also GABA mediated and chloride dependent.

Figure 26B is an example of the IPSP's dependence on extrinsic current (and resulting membrane potential). Measurements of the transient light-on response, made during various levels of extrinsically applied current (allowing the membrane potential to reach steady state), are plotted in Figure 27. The IPSP is linearly related to applied current over the entire range measured. This suggests that, at least in the hyperpolarizing direction, it is also linearly related to the membrane potential. Unfortunately, there is no way of determining the membrane potential or absolute size of the IPSP at the site of its synaptic generation.

D. DISCUSSION

The neurosecretory cells of the MEXO possess a modality specific (visual) synaptic input. This is a novel observation for neurosecretory cells in invertebrates as well as vertebrates (Mason and Bern, 1977). The only other study published to date that provided direct evidence of a modality specific synaptic influence on a neurosecretory cell was in the in vitro rat hypothalamic slice preparation (Mason, 1980).
Current steps of known values were applied to cell bodies in two different preparations. The membrane potential was allowed to reach steady state and then a light pulse was applied to the cornea within the impaled cell's receptive field. The data points represent averages of at least two light responses at each current level. The line is a least-squares linear regression fit with $r=\pm 0.99$. Measurements were taken from the peak of the transient phase of the IPSP.
FIGURE 27
Mason (1980) presented data that indicated the supraoptic neurons of the hypothalamus are themselves osmosensitive, and that apparently their response to an osmotic stimulus is passed to neighboring supraoptic neurons via direct synaptic connections. The MEXO cells, rather than being primary transducers of the stimulus energy (light) that ultimately affects their activity, are interneurons. By virtue of the fact that they receive synaptic input from the retinotopic projection originating with the photoreceptors in the retina, they are visual interneurons.

The MEXO cells also satisfy several important criteria which distinguish neurosecretory from nonneurosecretory neurons. The characteristics of the MEXO cells include: 1) They possess large cell bodies which appear blue-white in fresh tissue and at the light microscopic level resemble Type I neurosecretory somata in the crayfish eyestalk described by Shivers (1967). 2) They have swollen axonal terminals which branch diffusely in a well vascularized area (the Lamina Ganglionaris). 3) Their somata are actively invaded by action potentials of extremely long duration. 4) They receive a sluggish (slow and long lasting), modulatory synaptic input capable of influencing their activity over prolonged periods of time. It is left to be determined whether they possess dense core neurosecretory vesicles which are released non-synaptically and have an effect on a distant target organ.

The neurohormonal role of the MEXO is still open to speculation. In the prawn, extirpation of the MEXO causes
the distal retinal pigment to freeze in its day adaptation (dispersed) position (Bellon-Humbert et al., 1981). This result combined with the synaptic inhibition to light-on reported here, suggests that the MEXO is involved; a) in the dark adaption migration of visual screening pigments (Fingerman, 1957), or b) in the circadian rhythm of visual screening pigment migration (Arechiga, 1977). These hypotheses are also consistent with the presumed release sites of the MEXO neurohormone, in the LG, where it could have short range influence on the photoreceptor terminals. The blood flow in this region is rapid and could carry the released substance distally to the retina or to the distal pigment cells of the ommatidia.

No excitatory input to the MEXO cells was observed. Spontaneous spiking appeared from unstable depolarizing shifts in membrane potential with no obvious synaptic contribution. It is possible that any excitatory influence may be a blood-born substance such as serotonin or octopamine (Evans et al., 1976). It should be noted that the release of hyperglycemic hormone and erythrophore-dispersing hormone is increased after injecting serotonin into intact animals. Release of neurodepressing hormone (NDH) also occurs following exposure of the sinus gland to serotonin (Arechiga, 1982). However, in my preparations the heart is removed and the haemolymph is not moving, therefore, no possible blood-born excitant would be able to reach the MEXO.

The branching pattern of the MEXO cells is not similar
to any of those presented by Nassel (1977) or Strausfeld and Nassel (1981). Contrary to what is believed to be the case for the MEXO in other decapods (Bellon-Humbert et al., 1981), the lucifer yellow fills of crayfish MEXO cells revealed no processes entering the sinus gland.

Only in two cases was dye-coupling present between MEXO cells. One case is illustrated in Figure 21C. A single MEXO cell passed dye to an entire cluster of small cell bodies. The coupled cell bodies did not possess any obvious processes within the neuropil, only extremely fine branches within the soma cluster. It seems plausible that these cells are precursor MEXO cells and that the dye-coupling is only present during particular synthetic stages of the MEXO cells. This would resemble the appearance and disappearance of dye-coupling between precursor DUM neurons during development in the locust (Goodman and Spitzer, 1979). Alternatively, the coupling could be due to surrounding glial cells. The other case of dye-coupling was to cells located slightly more ventral to but apparently a part of the MEXO.
REFERENCES


