INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.

University Microfilms International
300 N. Zeeb Road
Ann Arbor, MI 48106

8319754

Coleman, Paul Anthony

MORPHOLOGICAL AND PHYSIOLOGICAL STUDY OF THE INTERACTIONS BETWEEN EXCITATION AND INHIBITION IN MUDPUPPY INNER RETINA

City University of New York PH.D. 1983

University
Microfilms
International 300 N. Zeeb Road, Ann Arbor, MI 48106

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark __√__.

1. Glossy photographs or pages __✓__
2. Colored illustrations, paper or print _____
3. Photographs with dark background __✓__
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy __✓__
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages __✓__
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages __✓__
15. Other _____

University
Microfilms
International

MORPHOLOGICAL AND PHYSIOLOGICAL STUDY OF THE INTERACTIONS BETWEEN EXCITATION AND INHIBITION IN MUDPUPPY INNER RETINA

by

PAUL COLEMAN

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1983

Coleman

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

4/25/1983
date

Chairman of Examining Committee

May 2 1983
date

Executive Officer

Supervisory Committee

The City University of New York

Abstract

MORPHOLOGICAL AND PHYSIOLOGICAL STUDY OF THE INTERACTIONS BETWEEN EXCITATION AND INHIBITION IN MUDPUPPY INNER RETINA.

by

Paul Coleman

Advisor: Thomas E. Frumkes

Inner retinal neurons from *Necturus maculosus* were characterized by intracellular recording, receptive field mapping, current injection and by superfusing the following agents: gamma- Amino-n Butyric acid (GABA), glycine, their antagonists, and 2-amino- 4-phosphonobutyric acid (APB). They were also iontophoretically injected with Horseradish peroxidase (HRP) for study by flat mount analysis. These measurements were verified by radial sections or computerized reconstruction and rotation. Amacrine and ganglion cells were categorized as either ON, OFF, or ON-OFF units based on excitatory response components. Additionally, some ON amacrine cells were observed to have perikarya displaced to the ganglion cell layer.

The axonal terminations of ON bipolars, as well as the dendrites of ON amacrine and ganglion cells were within the

vitreal IPL, OFF amacrine and ganglion cells' processes projected toward the scleral IPL. Even though this difference between ON and OFF dendritic fields was statistically significant ($p<0.0005$), overlap was observed. ON and OFF IPSPs were observed in some amacrine and ganglion cells, however these inhibitory potentials did not adhere to the morphological lamination pattern observed for EPSPs.

The interactions of excitatory and inhibitory potentials were studied by restricting attention toward ON cells whose photic response is dominated by a sustained depolarization, an initial burst of spikes and a homogeneous receptive field. These cells have different types of inhibitory inputs. In some cases both ON and OFF IPSPs were blocked by picrotoxin. For others, picrotoxin attenuates the ON IPSP and strychnine the OFF IPSP. Finally, strychnine causes the depolarization from the dark potential by 5-10 mV and attenuates the light evoked depolarization in some cells. These results suggests interactions with GABAergic ON and glycinergic OFF interneurons.

The underlying basis for OFF IPSPs in ON cells is problematic since these phenomena contradict the apparent laminar organization for excitation. The possibilities considered include, overlap between ON and OFF sublaminae and/or neurons not yet identified in mudpuppy which terminate in the "wrong" area of the IPL. In either event, a OFF IPSPs in ON cells suggests a modulatory mode of action.

TABLE OF CONTENTS

	Page
List of Illustrations and Table	
I: General Introduction	1
A. Physiological Basis of ON and OFF Systems.	3
B. Anatomical Correlates of ON and OFF Systems.	5
C. Inhibitory Potentials of the Inner Retina.	6
D. Rationale for Present Study.	7
II: Methods	10
A. Subjects.	10
B. Dissection.	10
C. Superfusion.	12
D. Photic Stimulation.	14
E. Recording.	15
F. Intracellular Staining.	16
III: Survey of Mudpuppy Inner Retinal Neurons	23
A. Introduction.	23
1. Morphological Description of Mudpuppy Bipolar, Amacrine, and Ganglion Cells.	23
a. Bipolar Cells.	24
b. Amacrine and Ganglion Cells.	25
2. Physiological Characterization of Mudpuppy Bipolar, Amacrine, and Ganglion Cells.	28
a. Bipolar Cells.	28
b. Amacrine Cells.	28

c. Ganglion Cells. 30

B. Methods. 34

C. Results. 36

1. Anatomical Overview. 36

2. Ganglion Cells. 38

a. General Anatomical Properties. 38

b. ON-OFF Ganglion Cells. 39

c. ON Ganglion Cells. 42

d. OFF Ganglion Cells. 43

3. Amacrine Cells. 44

a. General Anatomical Properties. 44

b. ON-OFF Amacrine Cells. 45

c. ON Amacrine Cells. 47

d. OFF Amacrine Cells. 49

D. Discussion. 103

IV: Functional Lamination of the Inner Plexiform Layer of the Mudpuppy Retina. 109

A. Introduction. 109

B. Methods. 113

C. Results and Discussion. 116

1. Quantitative Observations. 116

2. Photographic Visualization. 118

3. Radial Sectioned Data. 119

4. Bipolar Cells. 120

V: Interactions of ON and OFF Potentials in ON Amacrine and Ganglion Cells. 132

A. Introduction. 132

B. Methods. 136

C. Results and Discussion. 137

VI: General Discussion. 149

A. Organization of Inhibition Within the Inner Retina: Insights Derived from the Study of ON Cells. 151

Appendix. 156

I. Naka's Model. 156

II. Werblin's Model. 158

III. Miller and Frumkes' Model. 161

Bibliography. 164

LIST OF ILLUSTRATIONS AND TABLE

Figure		Page
1	Schematic of the 2-channel optical system used in the present study.	21
2a	Synaptic organization of the mudpuppy retina.	32
2b	Samples of intracellularly recorded responses from photically stimulated retinal neurons.	32
3	Light micrograph of the mudpuppy retina in a 1 *um* thick radial section showing the nuclear and plexiform layers.	53
4	Light micrograph of a quadrant of flat mounted mudpuppy retina, stained by retrograde transport of Horseradish peroxidase.	55
5	Camera lucida drawings of two ON-OFF ganglion cells.	57
6	Camera lucida drawings of three ON-OFF ganglion cells.	59
7	Photomicrograph of the flat mounted, ON-OFF ganglion cell illustrated in Figure 5a.	61
8	Photomicrograph of a ganglion cell that only demonstrated transient ON-OFF IPSPs.	63
9	Receptive field of an unstained ON-OFF ganglion cell.	65
10	Camera lucida drawings of two ON ganglion cells.	67
11	Camera lucida drawings of two ON ganglion cells.	69
12	Photomicrograph of the flat mounted, ON ganglion cell illustrated in Figure 10a.	71
13	Camera lucida drawings and intra-cellular responses of two OFF	73

	ganglion cells.	
14	Photomicrograph of the flat mounted, OFF ganglion cell illustrated in Figure 13b.	75
15	Camera lucida drawing and intracellular response of an ON-OFF amacrine cell.	77
16	Camera lucida drawing and intracellular response of an ON-OFF amacrine cell.	79
17	Camera lucida drawings and intracellular responses of two ON-OFF amacrine cells.	81
18	Photomicrograph of the flat mounted, ON-OFF amacrine cell illustrated in Figure 17a.	83
19	Photomicrograph of a flat mounted, amacrine cell.	85
20	Receptive field of an unstained ON-OFF amacrine cell.	87
21	Camera lucida drawings and intracellular responses of 4 ON amacrine cells with conventionally located perikarya in the inner nuclear layer.	89
22	Photomicrograph of the flat mounted, ON amacrine cell illustrated in Figure 21d.	91
23	Camera lucida drawings and intracellular responses of 3 displaced ON amacrine cells.	93
24	Photomicrograph of the flat mounted, displaced ON amacrine cell illustrated in Figure 23c.	95
25	Receptive field plots of an unstained ON cell and the cell represented in Figure 23a.	97
26	Camera lucida drawings and intracellular responses of 2 OFF amacrine cells.	99

27	Photomicrograph of the flat mounted, OFF amacrine cell illustrated in Figure 26b.	101
28	Graph demonstrating the spatial density of the dendritic fields of 12 ON and 4 OFF cells.	122
29	Bar graph showing the extremes of the dendritic fields of the cells in table 1.	124
30	Light micrograph of three ganglion cells shown at two different planes of focus 14 *um* apart.	126
31	Light micrograph of an HRP stained ON amacrine cell from a radially sectioned retina.	128
32	Camera lucida drawing of depolarizing bipolar cell and computer reconstruction.	130
33	Intracellular responses from a displaced ON amacrine cell.	143
34	Intracellular responses from a displaced ON amacrine cell.	145
35	Intracellular responses from an unstained ON cell.	147
Table 1		51

Chapter I

General Introduction

Visual information is conveyed from the vertebrate retina to the brain by the axons of single ganglion cells (optic nerve fibers) in the form of all or none spikes or action potentials. Since the early work of Hartline (1938) the information conveyed by a ganglion cell has often been summarized by describing the cell's receptive field, e. g. that area of the retinal surface which when photically stimulated elicits a change in response of the recorded cell.

The description of ganglion cell receptive fields is most often carried out by recording extracellular spikes from single cells and the best known categorization stems from the work of Kuffler (1953). He labeled units that increased their rate of firing, above dark baseline levels, when photically stimulated within a restricted area close to the recording electrode as "ON" center cells. There were other units that decreased their firing rate below baseline levels, when stimulated in a similar way; these were called "OFF" center cells. Stimulation of the surrounding retinal area elicited a response opposite or antagonistic to the effects of center stimulation. Although rare in cat, many other vertebrates display an additional class of ganglion cells that respond at the initiation and termination of the

photic stimulus. These are "ON-OFF" cells, which respond maximally to small stimuli in the center of their receptive fields (Barlow, 1953).

Since 1953, many studies have attempted to extend knowledge of retinal functioning by means of extracellular single unit recording. Thus, a good deal of data has documented the influence of light and dark adaptation, retinal position, movement, and chromatic variables in determining the receptive field properties of ganglion cells (Rodieck, ch. 19, 1973). Probably the most wide reaching finding was the further division of mammalian retinal ganglion cells into X-, Y-, and W- subcategories (Enroth-Cugell and Robson, 1966; Stone and Hoffman, 1972). Overall, such data have been valuable in the formation of modern theories of vision which interrelate ganglion cell function to receptive field properties of cerebral neurons (Hubel and Wiesel, 1968; Stone, Dreher and Leventhal, 1979; Lennie, 1980) or psychophysically defined visual channels (Mahr, 1982). The present study views the ganglion cell response as the end point of interest and is concerned with the antecedent synaptic events within the retina which can be only studied by intracellular recording.

Physiological Basis of ON and OFF Systems.

The first functional study of the overall intrinsic organization of the retina was that of Werblin and Dowling (1969) in mudpuppy. After recording intracellular responses from the major classes of retinal neurons, they iontophoretically injected Niagara blue dye into the cell to permit morphological identification. Their findings, when combined with electron microscopy (EM) and Golgi studies (Dowling and Boycott, 1966; Dowling and Werblin, 1969) led to a model of retinal functioning which could interrelate the anatomical findings of Cajal (1893) and the extracellular spike findings of Kuffler (1953). The essentials of their findings were soon replicated in other species (Kaneko, 1970; Schwartz, 1974; Naka and Ohtsuka, 1975) and hence, have provided the foundation of more modern descriptions of retinal organization (Naka, 1977; Dowling, 1979; Wunk and Werblin, 1979; Frumkes, Miller, Slaughter, and Dacheux, 1981). In its most basic form, the Werblin - Dowling model describes the flow of neural information through the retina as a radial pathway from photoreceptors to bipolar to ganglion cell. Two functional classes of bipolar cells were observed. One type of these non - spiking cells depolarizes in a graded fashion with stimulation of its receptive field center, presumably due to direct photoreceptor influence. The

other type hyperpolarizes to stimulation of the receptive field center. For both cell types, stimulation of the surround would evoke a response of the opposite polarity presumably due to interceding horizontal cells. Werblin and Dowling hypothesized that ON and OFF center ganglion cells, as described by Kuffler, could be accounted for by the depolarizing and hyperpolarizing bipolar cells. They did not specify, however, which class of bipolar was presynaptic to which type of ganglion cell, nor did they identify any morphological differences between neurons of the ON or OFF systems.

More recent studies have established that depolarizing and hyperpolarizing bipolar cells provide, respectively, the excitatory input to ON and OFF ganglion cells. For example, Naka (1977) has electrically stimulated a bipolar cell while simultaneously recording from nearby ganglion cells. Injecting positive current into a depolarizing or ON bipolar cells excites ON ganglion cells, but has no influence on OFF ganglion cells. He similarly showed an excitatory relationship between hyperpolarizing or OFF bipolars and OFF ganglion cells. Other physiological demonstrations include the comparison of thresholds and latencies (Frumkes, and Miller, 1979); ion substitution experiments (Miller and Dacheux, 1976; Dacheux, Frumkes and Miller, 1979) and experiments with pharmacological agents that affect the excitatory amino acids of the retinal network (Slaughter and Miller, 1981;

1983).

Anatomical Correlates of ON and OFF Systems.

As first established by Cajal (1893) using silver impregnation techniques, the neuropil of the inner plexiform layer is arranged into 5 strata. Famiglietti and Kolb (1976) collapsed these 5 strata into 2 separate sublaminations based on the functional characteristics of ON and OFF. Cajal's strata 1 and 2 (toward the sclera) are Famiglietti and Kolb's lamina A; the remaining three (toward the vitreous) are lamina B. In the cat, these two functional laminations are separated by a bed of capillaries. Cells associated with the ON system project their neurites to lamina B; the OFF system to lamina A (Famiglietti and Kolb, 1976). This was confirmed using intracellular injections of Procion yellow and "lucifer" dyes (Famiglietti, Kaneko, and Tachibana, 1977; Nelson, Famiglietti, and Kolb, 1978). In addition to ganglion cells, this functional stratification also includes bipolar and amacrine cells. Although the number of species is increasing in which the functional stratification of the inner plexiform layer is found (Bloomfield, 1981), exceptions to this schema are also becoming more numerous (Davies and Naka, 1980; Weiler and Marchiafava, 1981).

Inhibitory Potentials of the Inner Retina.

Intracellular recording from ganglion cells also demonstrates the presence of inhibitory potentials, most commonly in the form of transient inhibitory post synaptic potentials (IPSPs) at light onset and offset (Werblin and Copenhagen, 1974; Miller and Dacheux, 1976). The best recognized source of ganglion cell inhibition in mudpuppy is the ON-OFF amacrine cell (Werblin, 1977; Miller, Dacheux, and Frumkes, 1977). As evidenced by the patterns of IPSPs observed in ganglion cells, of which several types have been found by separate groups of investigators, the ON-OFF amacrine cell cannot be the sole source of inhibition within the inner plexiform layer (Frumkes, Coleman, and Nicotera, 1980; Frumkes, et al., 1981; Belgum, Dvorak, and McReynolds, 1982). ON and OFF amacrine cells have been anatomically identified in fish (Kaneko, 1970; Naka and Ohtsuka, 1975), turtle (Weiler and Marchiafava, 1981), rabbit (Bloomfield, 1981) and cat (Nelson, 1982). No one has yet established, however, whether these cells function in an inhibitory and/or excitatory manner. Another possible source of inhibition is from a class of bipolar cells that have been found to take up glycine (McGuire, Stevens, and Sterling, 1980); suggesting the existence of inhibitory bipolars.

Rationale for Present Study

Most current models of the vertebrate retina posit that the general response properties of ganglion cells can be understood largely in terms of their excitatory bipolar input. Hence, ON, OFF, and ON-OFF ganglion cells are driven by the ON, OFF, or both bipolar types. Detailed analysis of IPSPs suggest additional parameters for the categorization of the three basic ganglion cell types, in recognition of the fact that the output message provided by ganglion cells is modulated by inhibition as well as excitation. Most investigators stress that in addition to a sustained excitatory post synaptic potential (EPSP) from bipolars, many ganglion cells display transient ON-OFF IPSPs. These IPSPs can be attributed to gamma- aminobutyric acid (GABA) or glycine releasing ON-OFF amacrines, or distinct ON and OFF inhibitory neurons (Frumkes, Coleman, and Nicotera; 1979, 1980; Frumkes, et al., 1981). This has led to a classification of ganglion cell responses in terms of both their excitatory and inhibitory input (see Frumkes, et al., 1981).

As proposed by Kuffler (1953), the neural events that lead to a ganglion cell's response would be well described as a "push-pull" modulatory system, which he inferred from the plasticity of a cell's receptive field. Detailed intracellular studies of the post synaptic potentials of ganglion

cells have produced data allowing such an interesting modification of the established retinal models. Belgum, et al. (1982) demonstrated that many ON ganglion cells are depolarized, up to 10 mV from their dark potential, by exposure of the retina to the presynaptic blocking agent Co++. They inferred that the light evoked, sustained EPSP is modulated by the dark release of an inhibitory transmitter substance. This source of dark inhibition could be from an OFF amacrine cell or a glycine releasing hyperpolarizing or OFF bipolar cell (McGuire, Stevens, and Sterling, 1980). These physiological observations indicate that some ON ganglion cells may be depolarized by a "push - pull" arrangement employing the combined effects of excitation and disinhibition; however, this interaction between ON and OFF systems presents a challenge to the generality of the principles associated with the functional lamination of the inner plexiform layer. A theoretical position strictly adhering to the structural segregation of ON and OFF systems could not explain the empirical findings of these OFF IPSPs in ON cells. But, examples of neurons that are in the "wrong sublamina", in particular those involved in the OFF system, have been recently documented (Zimmerman and Dowling, 1979; Weiler and Marchiafava, 1981; Nelson, 1982). It may be that these exceptions provide the OFF IPSPs in ON cells.

The present study attempts to interrelate the anatomical and physiological properties of neurons which synapse

within the inner plexiform layer of the mudpuppy retina by means of intracellular recording and staining with electrodes filled with Horseradish peroxidase. This approach was adopted for three reasons. First, although as much is known about the physiology of this retina as that of any vertebrate, anatomical study is very limited. The available Golgi and EM studies (Dowling and Werblin, 1979) and those using intracellular staining with Niagara blue (Werblin and Dowling, 1969) or "lucifer" (Zimmerman and Dowling, 1979) have not provided detailed information about dendritic structure and organization. These techniques were unable to reliably impregnate the fine or very distal dendrites of the mudpuppy retinal neurons. Second, although the action of various interneurons within the inner plexiform layer has been inferred, Werblin and Dowling (1969) only indentified the ON-OFF amacrine cell. The use of Horseradish peroxidase has allowed the identification of a wider variety of amacrine cell types. Third, the combined use of improved microelectrodes and superfusion makes it possible to obtain pharmacological, physiological, morphological data from the same retina, and in a few instances the same cell. The present study has obtained results confirming the bilaminar organization of excitatory input within the inner plexiform layer previously established in other species but at the same time, emphasize a more complex set of inhibitory relationships.

Chapter II

Methods

Subjects. All data were obtained from adult mudpuppies (*Necturus maculosus*) approximately 15-20 cm in length and obtained from commercial vendors. The animals were stored in aerated water - filled tanks. The temperature was kept at 4 degrees C. At irregular intervals they were fed live goldfish approximately 3 cm in length. Except where noted, the following procedures are modifications of those used by Miller and Frumkes (Miller and Dacheux, 1976; Miller, Frumkes, Slaughter, and Dacheux, 1981a).

Dissection. The animal was initially decapitated and double pithed. After removing the lower jaw, the head was bisected in the sagittal plane. One hemihead was saved in a moistened paper towel in a refrigerator for future experimentation. The front and back of the other hemihead was cut leaving approximately 2 cm of skull and muscle surrounding the eye. The eye was exposed with the removal of the remaining skin and the major muscles of the face were trimmed from the skull. The eye, which remained attached to the remaining skull and hard palate, was then placed on a Ringer-soaked cotton ball.

Using the dimmest illumination possible and with the aid of a dissecting microscope the cornea was opened, the

pupil slightly widened by cutting away some of the iris and the lens removed. With extreme care, the vitreous humor was drained off with absorbent tissue paper (Kim Wipes). This is a very crucial step in the dissection procedure and is particularly difficult if the vitreous is relatively viscous. On the one hand, electrode penetration is hampered, if too much vitreous remains. On the other hand, the retina may be partially or entirely detached from the pigment epithelium by attempting to remove more of the thick vitreous, in addition, waves of spreading depression may be initiated (Olsen and Miller, 1977).

If the eyecup was not superfused, it was left on the wet cotton ball and placed on a lucite and rubber stage which had a chlorided silver plate within to serve as an electrical ground. The superfused eyecups were draped with an apron fashioned from Kim Wipe tissue. The apron was approximately 1 cm in diameter with a center hole about 2 mm, slightly smaller than the diameter of the eyecup. After the apron was placed on the eyecup, the edges of the hole were tucked in around the remaining iris gently touching the retina. One or two fine tissue wicks were placed inside the bottom of the eyecup. The apron and wicks were important because they allowed smooth flow of the perfusate out of the eyecup, which in turn promoted exchange of fluid and prevents detachment of the retina. This preparation was taken off the cotton and placed on a plug made of hardened Ringer's agar and placed on the lucite stage with the

chlorided silver ground.

The mounted eyecup was placed inside a light tight Faraday cage on a bidirectional slide and the photic stimuli were aligned over and focused on the retina. For the dry eyecup, moistened 100% oxygen was blown over the preparation. Temperature, within the Faraday cage, was maintained at approximately 25 degrees C with 2 liters (plastic soda bottle) of ice placed near the preparation.

Superfusion. The superfused preparation was slightly more complicated than the dry eyecup in that delivery and drainage of the perfusate had to be accommodated. The oxygenated perfusate was delivered through a 1.2 mm capillary pipette fashioned so that its output was approximately 3cc/min. In experimental situations, however, the tip of this pipette was placed in the upper half of the eyecup so that the flow of the perfusate was continuous. Drainage was through a system of plastic tubes, hoses, and tissue wicks; arranged so that there was a smooth flow from the retina to the collecting reservoir (drips cause massive electrical artifacts). Temperature was maintained at 23 C or less, by placing 2 liters of ice in a compartment of the cradle holding the bottles storing the perfusate. Superfusion was accomplished by means of a gravity feed apparatus. A separate, electrically grounded Faraday cage was hung over the experimental chamber and contained a cradle with eight 250 ml reservoir bottles. Each bottle had its own valve, which was connected to 18 ga teflon tubing.

The 8 separate teflon tubes were wrapped together, electrically shielded, and fed into the experimental chamber (a light tight Faraday cage). The tube from each bottle was connected to a mixing chamber, the output of which was brought to the retina through a five cm piece of one mm (inner diameter) rubber tubing and capillary pipette. Exchange of fluids occurred in less than 30 sec and rarely disrupted the intracellular record with either physical or electrical artifacts. All the bottles were aerated with 100% oxygen.

Each bottle contained a separate solution, all of which were modifications of the Ringer's solution for the mudpuppy retina described by Miller and Dacheux (1976). All solutions were made with distilled and demineralized water.

The basic Ringer's solution was:

111.0 mM NaCl	3.0 mM KCl
1.8 mM CaCl2	1.0 mM MgCl
3.0 mM Hepes (Sigma H-3375)	11.0 mM glucose

Five of the solutions were made by adding appropriate pharmacological agents or salts to basic control Ringer's:

2 - 5 mM gamma-Amino-n-Butyric Acid (Sigma A-2129)
10 - 50 uM Picrotoxin (Sigma P-5753)
2 - 5 mM Glycine (Sigma G-7126)
10 - 50 uM Strychnine Sulfate (Sigma S-7001)
20 - 100 uM 2-Amino-4-Phosphonobutryic Acid (Calbiochem 165303)

Although results were occasionally obtained with other perfusates, these data were not included. All solutions

were titrated to a pH of 7.8 with a solution of 5% NaOH

The pH of all solutions were checked before the experimental session. Solutions were made every 7-10 days. This was to avoid excessive Na+ concentrations due to adjustment of pH; also to insure against biological contamination. At the end of the working day, the bottles were capped with rubber stoppers and stored at 4 degrees C in a refrigerator.

Photic stimulation. Photic stimulation was accomplished by means of a 2 channel optical bench as represented in fig 1. Each beam was derived from separate, tungsten-halogen sources (Atlas 100 W). This is a 12 volt source, but was intentionally underpowered at 11.5 volts to preserve bulb life. The maximal source intensity was found to be 2.25 uW/cm2 at 577 nm and was attenuated by two, 4-log unit circular neutral density wedges geared in counter rotation to provide 8 log units of continual intensity adjustment. In addition, infrared illumination was filtered out of each beam. The standard illumination employed was white light, attenuated 3 to 4 log units from maximum. Stimulus duration was controlled by electromagnetic shutters (Uniblitz) driven by an electronic timer (Ortec).

The spatial configuration of one stimulus was controlled by a series of concentric circular apatures which ranged from 0.2 mm to 3.0 mm. The other channel provided a 0.1 mm wide slit which could be swept across the retina in

0.013 mm increments. Usually, each stimulus channel was presented for 2 sec, once every 10 sec.

Recording. Intracellular electrodes were fashioned from thin wall (0.33 mm), triangle shaped glass tubing (1.25 mm outer diameter, Glass Company of America). This tubing has the quick filling property of fiber filled tubing. In addition, these electrodes have lower resistances while maintaining their linear properties over a larger current range than a comparable sized electrode made from fiber filled glass. The electrodes were formed on a vertical puller (Narashige) and filled with 0.5 M potassium acetate, buffered at pH 7.6 using 0.05 M Trizma hydrochloride (Sigma T-3253), and backfilled with 2 M potassium chloride. In the dry eyecup electrode resistances ranged from 80 to 150 megohms. Higher resistance electrodes were needed in the superfused retina; they ranged from 120 to 300 megohms. In general the electrode was dipped briefly into a 2% solution of dichloro-dimethylsilane (Eastman Kodak 9650) in carbon tetrachloride, a technique suggested by R.F. Miller (personal communication). This is presumed to form a hydrophobic coating presumably aiding the lipid bi-layer constituting the cell's membrane to better seal around the electrode.

Intracellular potentials were amplified through a high input impedance, D.C. coupled amplifier with current injection capabilities (WPI model 701). Its output was split and fed into a digital voltmeter providing a continuous,

easy to view monitor of membrane potential and into one channel of a rectilinear penwriter for a permanent record (Beckman Offner type S Dynagraph). This output was also sent to a differential offset amplifier, which allowed compensation for the cell's standing membrane potential so that it could be easily viewed at higher gains on a storage oscilloscope (Tecktronics) and penwriter.

The ground reference from the preparation, which completes the amplifier's recording circuit, was interrupted by an in-line current monitor (Analog Devices, AD 515). The sensitivity was adjusted to -1 pA/mV. This device allowed a true reading of the electrode's current output into the preparation and additionally warned against artifactual potentials due to unprotected salt to metal contacts.

Under microscopic guidance (Nikon), the electrodes were positioned over the preparation with a micromanipulator (Narashige). The electrodes were advanced through the retina, with a manually operated hydraulic microdrive (Narashige), in small steps (less than 0.01 mm) until a sudden negative shift from ground occurred. To assist the impalement of neurons, the preparation was quickly "buzzed" by sending the amplifier's capacitance compensation adjustment into oscillation. This caused a train of high frequency +1 na pulses to be emitted through the electrode.

Intracellular staining. Following impalement and physiological study, many cells were "filled" with

Horseradish peroxidase (HRP). These cells were visualized using the chromogen di-amino benzidine (DAB). The chromogen tetra methyl benzidine (TMB) was also utilized, but soon phased out. The DAB reaction was favored for 2 reasons: first, DAB is less prone to various artifacts and gives the stained neuron a better sense of definition than TMB. Second, neurons reacted with DAB can better withstand further histological processing than other chromogens (Bloomfield, 1981). The Benzidine di-hydrocholoride reaction was not used since proper facilities were not available for handling this powerfully carcinogenic agent.

Iontophoretic injection of HRP was accomplished by filling the tip of the electrode with a 10% solution of HRP (Boehringer-Manheim, Grade I) in 0.5 M potassium acetate, 0.05 M Trizma HCL Buffer at pH 7.6. The rest of the electrode was filled with 0.5 M potassium acetate and backfilled with potassium chloride. The actual concentrations of the solutions is not as important as the relative concentrations between the HRP and the potassium ion. One must use the lowest concentration of salts and the highest concentration of HRP possible; otherwise the smaller potassium ion will be injected into the cell rather than the desired HRP.

Various current parameters were used for HRP iontophoresis. With the HRP buffered at pH 7.6, positive current is needed to "push" it out of the electrode. The usual current parameters were a $\pm$ 0.5 na 3 Hz sine wave

modulated about ground for 10 min; but for faster filling (less than 3 min), D.C. current or brief pulses (30 msec with 50% duty cycle) was used. For any given cell, the favored current parameters were that which filled the fastest (without disrupting cell membranes), maintained a fairly linear relationship between current and voltage, and did not saturate the amplifier by producing an IR change of greater than 1.75 volts in respect to ground.

Another method of discharging HRP, which was very undesirable, was to leave the capacitance compensation in oscilation more than necessary. Overuse of this feature would cause artifactual staining of extracellular space which would allow uptake of HRP by Muller cells and could lightly stain unimpaled neurons. These cells can be distinguished by the use of dark field microscopy, since only well filled neurons are clearly seen. If more than one neuron was stained in a retina, a map of electrode penetrations was sketched to insure accurate location.

After HRP injection the eyecup was left 15-30 min in the dark, which was sufficient time for the HRP to travel (Dacheux, personal communication), and for the pigment epithelium to recede back from around the photoreceptors (Stone, 1981). In cold Ringer's, retina was then disected from the eyecup, mounted on a piece of membrane filter (Gelman, 0.45 um pore size), immersed in cold fixative (1.5% paraformaldehyde, 1.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3) for 15-20 min and then washed

overnight in 0.1 M phosphate buffer, pH 7.3 which was changed once the next morning.

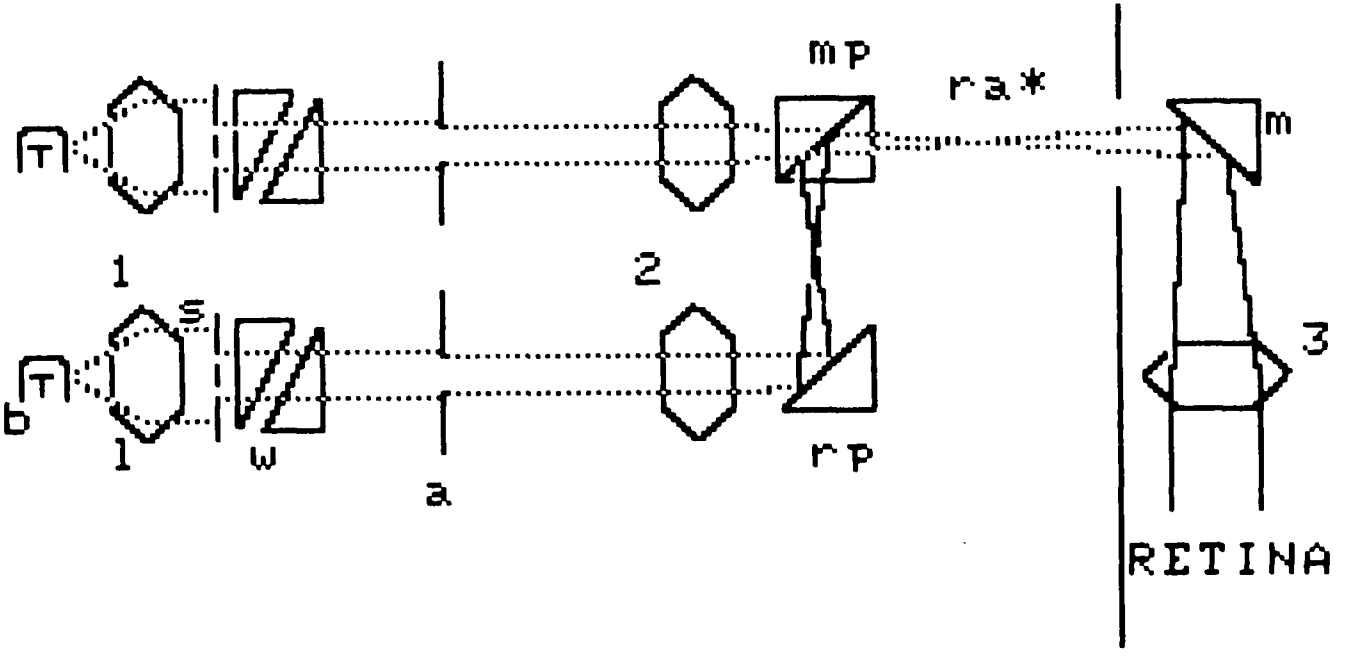
The HRP was reacted by first incubating the preparation in 0.05% solution of 3,3'-diaminobenzidine tetrahydrochloride (Sigma, D-9015) mixed in 0.05 M Trizma HCL pH 7.6 for 30 min. The retina was then transferred to another container, for another 30 min, with the same DAB solution, but with 0.2 ml of 0.3% H202 / 10 ml of buffer. Following the completion of the reaction the retina, was washed 3X in phosphate buffer, dehydrated with ascending alcohols, cleared in xylene and mounted whole on a slide (vitreal surface up) with Coverbond (Fisher). In a few instances, the whole retina and some stained cells were measured before and after dehydration. There was no more than a 10 to 15% reduction due to shrinkage. All procedures involving the use of DAB were done under a laboratory hood, with protective gloves, mask, eye goggles and gown. Anything contaminated by the DAB was soaked in sodium hypochlorite (household bleach) and disposed in an appropriate manner.

If the retinas were to be sectioned, they were osmicated, dehydrated by ascending alcohols, infiltrated with increasing concentrations of Epon Araldite in propylane oxide and flat mounted between two plastic cover slips. Upon completion of whole mount analysis, the cell to be sectioned was removed from the rest of the retina by cutting through the plastic cover slips with a scalpel. The chip

containing the cell was glued to a blank block of Epon, with 5 Min epoxy. The mounted retina was radially sectioned at approximately 6 um until it was estimated that all labeled dendrites were taken.

Figure 1. Schematic of the 2 - channel optical system used in the present study. It can be divided into three stages. At the first stage (1) the source (b) is collimated by the first lens (l). Due to physical restraints, the shutters (s) and neutral density wedges (w) are inserted at this early stage. The aperture (a) is placed 1 focal length (400 mm) from the lens of the second stage (2). Between the aperture and the second lens, infrared or chromatic filters are placed.

The beam is then deflected 90 degrees by a reflecting prism (rp) and is combined with the beam of the upper channel in a mixing prism (mp). Before the combined light beams can enter the light tight experimental chamber, it must be deflected 90 degrees with another reflecting prism at point ra*. At the last stage (3), the light is directed to the preparation with a mirror (m) and recollimated by the final lens, which can be adjusted to focus the image of the aperture on the retina. Matte black baffles were placed at necessary locations to limit stray light.


mp
ra*
m
1
2
3
s
b
l
w
a
rp
RETINA

Chapter III

SURVEY OF MUDPUPPY INNER RETINAL NEURONS

Introduction

Morphological Description of Mudpuppy Bipolar, Amacrine, and Ganglion Cells

Figure 2a is a drawing of a radial section of the mudpuppy retina and shows that its organization is typical of that of other vertebrates. Based upon their measurements, Dowling and Werblin (1969) indicated that perikarya of mudpuppy retinal neurons were about 25-30 *um* in diameter. The cell bodies of this laminated structure are arranged into three layers, separated by two plexiform layers of dense neuropil. The ganglion cell layer, usually only one cell thick in mudpuppy, contains the output neurons of the retina. The inner nuclear layer contains a heterogeneous collection of neurons and glial cells, consisting of amacrine, bipolar, horizontal cells, and Muller cells, the principal type of glial cell. The outer nuclear layer contains the cell bodies of the rods and cones.

The nuclear layers are separated by the two plexiform layers. The outer plexiform layer, which is thin and irregular in mudpuppy, contains the processes of the receptors,

horizontal and bipolar cells. Recently, neurons have been identified that send processes to both outer and inner plexiform layers. These are the interplexiform cell (Dowling, 1979) and the biplexiform cell (Zrenner, Nelson, and Mariani, 1982); however, they have not been identified in mudpuppy. In the course of data collection for the present study, neurons of the outer retina were stained but the following is restricted to the consideration of the bipolar, amacrine, and ganglion cells that synapse within the inner plexiform layer.

Bipolar Cells

The bipolar cell is the primary interneuron between photoreceptors and ganglion cells. The bipolar cells of the mudpuppy retina are rather homogeneous in apperance (especially since there is no fovea). These bipolars usually possess a Landolt club, which is a process that emanates from the center of the cell's dendritic field and can project between receptor cells and are reported to lack synaptic contacts. Landolt clubs are usually found on smaller types of bipolars and their functional significance is not known (Rodieck, 1973, p. 384). Mudpuppy bipolars are postsynaptic to both receptor types and horizontal cells. These interconnections occur mostly within the outer plexiform layer. Occasionally bipolars are contacted by photoreceptors and

horizontal cells directly on their perikarya. In addition, horizontal cells synapse on bipolars within the triadic arrangement of the cone invagination (Dowling and Werblin, 1969). In tiger salamander, Lasansky (1980) reports the presence of presynaptic contacts from bipolars that feed into horizontal cells, but these are rare and have not been identified in other species.

The bipolar's axon terminates in the inner plexiform layer and synapses with amacrine and ganglion cells. The presynaptic profile of the bipolars includes both conventional and ribbon synapses. There are approximately four to five times as many conventional than ribbon synapses. Ribbon synapses are only found in bipolars within the inner plexiform layer. At the ribbon synapse, there are processes from at least two cells. This arrangement is called a dyad. In slightly more than half of the observed ribbon synapses in mudpuppy, both post synaptic processes are from amacrine cells. Many amacrine cell process also have presynaptic profiles at the bipolar ribbon synapse, indicative of a reciprocal relationship between bipolar and amacrine cells.

Amacrine and Ganglion Cells

The cell bodies of the amacrine and ganglion cells have been classified in a variety of ways depending on the morphological feature that a particular investigator wishes to

emphasize. These include: dendrite characteristics (Cajal, 1893; Polyak, 1941; Boycott and Dowling, 1969; Boycott and Wassle, 1974; Kolb, Nelson, and Mariani, 1981), cell body size and distribution (Cleland, Levick and Wassle, 1975; Wassle, Levick and Cleland; 1975) and functional characteristics (Naka, 1976; Nelson, Famiglietti and Kolb, 1978). Due to the limited number of morphological observations, the manner in which mudpuppy amacrine and ganglion cells fit into any of the above categorizations is unclear. The closely related tiger salamander has been better morphologically described (Wong-Riley, 1974; Vallegra, 1981), but is only recently receiving attention by physiologists interested in inner retinal function (Wunk and Werblin, 1979).

Ganglion cells have been classically distinguished from amacrine cells by the presence of an axon. Golgi impregnation, retrograde transport of stains, intracellular injection of stains, and antidromic stimulation have all occasionally proved unreliable as techniques for identifying axons (Naka, 1977; Parnas, 1979; Davies and Naka, 1980). Since some amacrine and ganglion cells are known to be "displaced", these observations suggest that any single criteria, particularly those based on negative evidence, are suspect as a means of identifying a neuron as an amacrine cell. Dowling and Werblin (1969) maintain that mudpuppy ganglion cell dendrites "often" appear less electron dense than those of bipolar and amacrine cells, when doubly stained with uranyl acetate and lead citrate and viewed with an

electron microscope.

The ultra structure of the inner plexiform layer of the mudpuppy has been very well documented (Dowling and Werblin, 1969). The synaptology from bipolars was already described, but to briefly summarize the connectivity, bipolars synapse on amacrine and ganglion cells with both conventional synapses and the specialized ribbon synapse. In addition to the connections between bipolar, ganglion, and amacrine cells, the inner plexiform layer is dominated by the interconnections between amacrine cells. Within the synapses of the inner plexiform layer, large granular synaptic vesicles, typical of catecholamine containing neurons, have been found in a few amacrine cells and never in bipolars.

Amacrine cells contact each other with either chemical or electrical synapses (Kolb and Famiglietti, 1974; Dowling, 1979; Naka and Christensen, 1981). The arrangements of the chemical synapses can be rather complex. Some processes of amacrine cells, where one side of a process has a post synaptic profile and the other a presynaptic profile, appear to be "stacked" in a serial arrangement. In addition to serial synapses, reciprocal synapses have also been observed between amacrine cell processes (Dowling, 1979). These anatomical arrangements are indicative of local circuits which do not involve the whole cell. The functional characteristics of these synapses have been hypothesized to play a role in motion selective attributes of some ganglion cells (Miller, 1979).

Physiological Characterization of Mudpuppy Bipolar, Amacrine, and Ganglion Cells

Bipolar Cells

Bipolar cells have been classified into two functional groups, based on their electrical response to a diffuse step of light: depolarizing and hyperpolarizing. The receptive field of both types is biphasic, in that it can be functionally divided into two concentric areas. Stimulation of the center produces the graded, non - propagating, response by which the cell is categorized. The polarity of the center response is opposed or antagonized with stimulation of the surround (Werblin and Dowling, 1969). In mudpuppy, the mechanism for center - surround antagonism is thought to result from the interaction between the bipolar and horizontal cells or feedback to photoreceptors. Figure 2b summarizes these relationships between retinal neurons and also provides examples of the intracellular responses.

Amacrine cells

ON-OFF amacrine cells have been observed in almost all species studied by means of intracellular recording. The response is characterized by a resting potential often more

negative than -60 mV and large transient EPSPs at both light onset and offset. These EPSPs can be as large as 30 mV in mudpuppy (Frumkes, et al., 1981). Also in mudpuppy, spikes of various amplitude, can be observed riding on these graded potentials (Miller and Dacheux, 1976d). Regardless of species, it is well accepted that ON-OFF amacrine cells have largely homogenous and monophasic receptive fields (Norman and Werblin, 1974; Naka and Ohtsuka, 1975).

Sustained amacrine cells have been reported in most vertebrate species (Kaneko, 1970, Naka and Ohtsuka, 1975, Bloomfield, 1981, Weiler and Marchiafava, 1981; Nelson, 1982). These cells have sustained post synaptic potentials that can be center depolarizing or hyperpolarizing. Although not observed in the earlier studies of the mudpuppy, Zimmerman and Dowling (1979) have stained one sustained ON amacrine cell. Nevertheless, others have encountered responses from cells with characteristics similar to ON amacrines, but with a Cl- dependent OFF IPSP (Dacheux, Frumkes, and Miller, 1979; Frumkes, et al., 1981). These sustained amacrine cells share many features with the more usual transient ON-OFF amacrines, e. g. photic response dominated by graded postsynaptic potentials and the maintenance of relatively deep membrane potentials. The receptive fields of these cells are usually large and monophasic; however like the transient amacrine cells (Werblin, 1970) when a moving slit approaches the periphery of their receptive fields there evolves a small transient response of the polarity,

opposite to that evoked by stationary center stimulation (Davies and Naka, 1981).

Ganglion cells

In the mudpuppy retina, ON-OFF ganglion cells are maximally responsive to stimuli between 500 and 650 *um* (Karwoski and Burkhardt, 1976). These cells display a transient response consisting of an EPSP - spike - IPSP sequence at both light onset and offset. The IPSPs are not always apparent when these cells are maintained near their normal dark potential (between -50 & -60 mV), but can be usually observed when the cell is depolarized either by injury or injection of positive current. In the majority of cases, all IPSPs are blocked by application of low concentrations of strychine or picrotoxin, suggestive of actions by glycinergic and GABAergic ON-OFF amacrine cells. These are further considered in Chapter V.

Many ON and OFF ganglion cells in the mudpuppy retina display center - surround antagonism with a receptive field center of between 500 and 750 *um* (Karwoski and Burkhardt, 1976). The membrane potential of ON ganglion cells tends to approach the lower values that are found in ON-OFF ganglion cells, with an average value of -56 mV. Conversely OFF ganglion cells are somewhat more depolarized with the average value remaining about -46 mV (Belgum, Dvorak and

McReynolds, 1982).

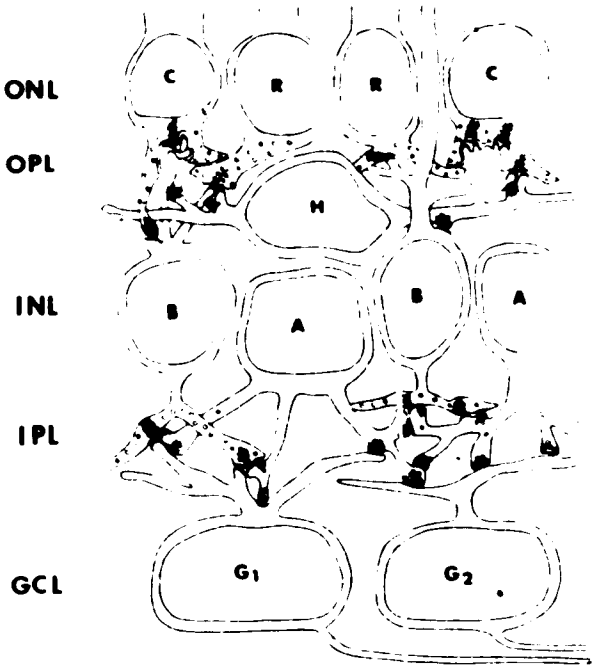
Recently, Frumkes, et al. (1981) have further categorized ganglion cells on the basis of their IPSPs. Some cells (sustained units) apparently receive little inhibition. A second class of cell, (transient type I) shows a GABAergic IPSP at light onset and none or little at offset. The final class of neurons (transient type II) show prominent IPSPs at light onset and offset, attributable to GABA or glycine releasing ON-OFF amacrine cells. In addition to the modulation of excitatory influences due to the center - surround antagonism of bipolar cells, spiking in ganglion cells is also governed by a lateral inhibitory mechanism of amacrine cell origin (Werblin and Copenhagen, 1974; Thibos and Werblin, 1978).

The present chapter involves data from 28 amacrine and ganglion cells of the mudpuppy retina. They were intracellularly recorded and stained with Horseradish peroxidase, which permits detailed observations of these neurons. Based upon their photic and morphological characterization, these results suggest a heterogeneity of amacrine and ganglion cell types as great as in any retina of other species.

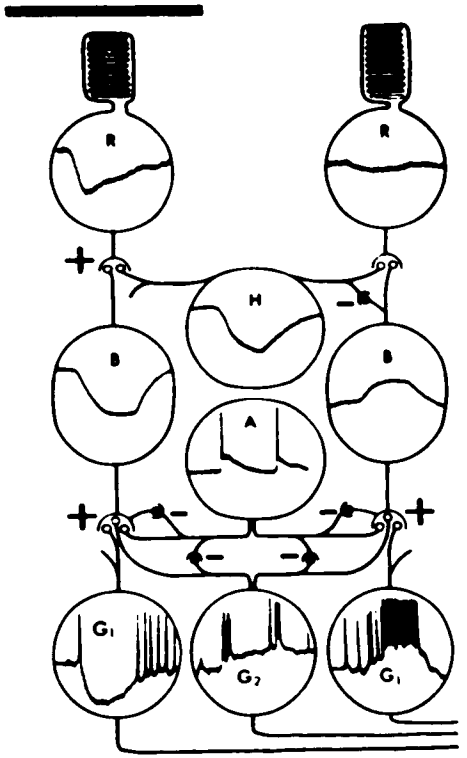
Figure 2a. Synaptic organization of the mudpuppy retina. Rods (R) and cones (C) contact horizontal (H) and bipolar (B) cells within the outer plexiform layer. Both types of photoreceptors contact these second order neurons through conventional and ribbon synapses; however invaginations, which contain processes from horizontal and bipolar cells (triad), are only found within cone terminals. Horizontal cells are presynaptic to other horizontal cells and to both hyperpolarizing and depolarizing bipolar cells. The bipolar cells are the vehicles from which information is transfered from the outer to the inner retina and are presynaptic to amacrine (A) and most ganglion cells (G). Other connections within the inner plexiform layer includes: contacts between amacrine cells, amacrines back onto bipolar cells, and amacrine to ganglion cells. (After Dowling and Werblin, 1969).

Figure 2b. Samples of intracellularly recorded responses from photically stimulated retinal neurons. They are oriented in their respective anatomical loci, with a schematic representation of the functional connectivity. (After Dowling, 1979). Bar represents photic stimulus.

2a



2b



Methods

In general, the procedures used were those outlined in the previous chapter. In addition, three other technical features will be described. First, in addition to intracellular injections of Horseradish peroxidase, some retinas were studied with more general stains. Cell bodies were stained with 1% toluidine blue suspended in 1% sodium borate in water (Kiernan, 1981). A drop of this solution was placed on a slide with radial sections of retina, which were embedded in Epon. The dye was drained off with absorbent paper and differentiated in acidic ethanol. In some cases, fresh retinas were immersed in a 5% solution of Horseradish peroxidase. By the action of retrograde transport ganglion cell axons were stained. Axons were also stained using the silver protargol method of Bodian (Biological Stain Commission, 1962) following standard histological procedures for the processing and sectioning of neural tissue embedded in parafin.

Second, the responses of some neurons were only obtained with diffuse illumination of the entire retina, while in other cases a cell's receptive field data were collected in one of two ways. According to the first technique, a small spot of light was centered around the recording electrode and the response to spots of different diameters (0.24 - 5 mm) were compared. In other cases, the receptive field was

mapped with a long (1 cm) slit of light and approximately 80 um wide. The receptive field size was estimated by moving the flashing narrow slit (for 2 sec cycled every 10 sec) across the retina in 65 um steps, until the entire receptive field was mapped.

Third, all anatomical data were obtained utilizing the flat mounted retina in order to minimize processing and to permit the full visualization of all neurites. This technique is gaining popularity in retinal neuroanatomy (for more details see Stone, 1981). The stained neurons were sometimes photographed with a camera mounted on a microscope (Nikon). Best results were obtained with blue light (480 nm) using either Kodak's Panatomic - X, or Technical Panchromatic black and white film; with a preference for the latter because of its variable contrast capabilities. In addition to or instead of photographic visualization, the stained cells were drawn using a camera lucida mounted on a microscope (Leitz). In virtually all cases a magnification of 1000X (oil immersion) was used. Generally, the camera lucida produced superior results because its depiction is not limited by the film's contrast detection properties or the len's depth of field which are problematic in conventional photographic procedures.

Results

Anatomical Overview

Figures 3 and 4 show the overall morphology of the mudpuppy retina. Figure 3 shows a radial or cross section of the mudpuppy retina stained with toluidine blue, and in essence, gives the same details noted by Dowling and Werblin (1969, e.g. as shown in figure 2a). Figure 4 is a photograph of a quadrant of an HRP stained flat mounted retina (vitreal surface up) that shows the collected ganglion cell axons as a system of fiber trunks that converge at the optic disk, at the center of the retina. Collectively, figures 3 and 4 provide a three dimensional representation of the retina. Figure 3 can be regarded as a thin slice, that is orthogonal to the plane of figure 4, which intersects at the ganglion cell layer.

Table 1 summarizes the anatomical information obtained from the bipolar, amacrine, and ganglion cells stained in the present study by means of intracellular injection of HRP. These cells were classified as ON, OFF, and ON-OFF units based on their photically induced excitatory potentials (EPSPs and spikes). The cell bodies of these inner retinal neurons were usually irregular in shape and ranged from 9 by 16 μm to 12 by 32 μm measured in a manner suggested by Stone (1981, p. 84). These values are consistent with results obtained by Zimmerman and Dowling (1979) using in-

tracellular injections of fluorescent stains. Collectively, these results indicate that the 25-30 *um* somata diameters reported by Dowling and Werblin (1969) may reflect a more homogeneous population of cells sampled by their employment of the Golgi procedure. The data from table 1 also show that dendritic fields vary in diameter from about 45 to 400 *um*. The larger value is greater than described in studies using other techniques, thereby supporting the assertion that intracellular injections of HRP is among the best techniques for complete filling of all cell processes (Friedlander, Lin, Stanford, and Sherman, 1981). The neurites of HRP stained cells varied considerably in diameter. Dendrites ranged from about 0.5 to 3.5 *um* in diameter, while ganglion cell axons ranged from the limit of resolution of light microscopy using X 1000 magnification (480 nm light, and an objective with a numerical aperture of 1.3) to 1.5 *um*. Axons were observed in ganglion cells that were intracellularly injected with HRP, and the retrograde transport by axons of HRP. In addition, similar results were obtained (though not illustrated) in retinae stained by the silver protargol technique of Bodian (Biological Stain Commission, 1962).

Ganglion Cells

General Anatomical Properties

The ganglion cells are the first neurons encountered, when the flat mounted retina is gradually brought into focus under the microscope. First the perikarya (within the ganglion cell layer) and then the dendrites (within the inner plexiform layer) are seen. The ganglion cell axons can be found at the most vitreal surface of the retina and usually this must be carefully. In some cases, an axon hillock can be seen protruding from the soma of the cell (figure 6a). Under high power magnification (X 1000) some axons can traced out of the general field and be seen to join other axons forming a network of thick (approximately 18 um) bundles, as shown in figure 3.

Examples of intracellularly stained ganglion cells are shown in figures 5-14 by means of camera lucida drawings and photomicrographs. The dendrites of these cells are smooth and are without any specialized appendages, like spines or stalks. The dendritic fields of these cells vary widely in appearance. Some can be described in terms of simple and deliberate patterns, while others are much more complex with the appearance of a roughly ripped piece of steel wool.

ON-OFF Ganglion Cells

Figures 5-8 display camera lucida drawings and sample intracellular responses from the ON-OFF ganglion cells described in this study. Unless otherwise indicated, all responses are to 2 sec duration, diffuse flashes of white illumination. The dendritic fields of ON-OFF ganglion cells varied in appearance. Three of the five ON-OFF ganglion cells encountered had dendritic fields which spread diffusely throughout the inner plexiform layer with many thin dendrites (figures 5a, 6a and 6b). Two other units (figures 5b, and 6c) displayed a much simpler dendritic field adhering to well defined planes within the inner plexiform layer.

All five of these cells displayed spikes at light onset and offset, although some variation was observed in the slower potentials that was not correlated with dendritic organization. Recent evidence demonstrates that the post synaptic potentials of ganglion cells are a mixture of excitatory and inhibitory influences (Frumkes, et al., 1981). Some cells readily displayed IPSPs, even though a relatively deep standing potential was observed. For other cells, evidence of transient inhibitory potentials can only be obtained if the unit is depolarized by injury or exogenous administration of positive current.

The ON-OFF ganglion cell of figure 6c displayed transi-

ent IPSPs at its observed resting potential. Application of GABA and glycine (2.5 mM) reversibly hyperpolarized and blocked the photic response. Application of picrotoxin (10 uM) blocked both ON and OFF IPSPs suggesting that these prominent inhibitory potentials were attributable to the action of a GABA releasing ON-OFF amacrine cell. In this cell, the effects of picrotoxin could not be reversed and hence, sensitivty to strychnine was not assessed. This cell had a simple, yet small dendritic field. Prominent ON-OFF IPSPs were also noted in the cell portrayed in figure 8. This unit's dendritic field was larger (300 um) than the cell of figure 6c and was diffusely structured, emphasizing the apparent lack of correlation between morphology and structure.

Because HRP exaggerates the rectifying properties of the intracellular electrode, current injection experiments using positive current to enhance IPSPs were only possible under the most favorable of circumstances. Of the two ON-OFF ganglion cells that did not display IPSPs when first impaled and could be depolarized with exogenous application of current (+0.5 nA), only one displayed any signs of transient inhibitory potentials (figure 5a). This neuron's dendritic field was very diffuse. The other cell showed no signs of inhibition and had a simpler dendritic structure, similar to the cell where the IPSPs were apparent.

The receptive field properties of ON-OFF ganglion cells have been studied in many lower vertebrates and all investi-

gators conclude that they respond maximally with photic stimuli 300 to 700 *um* in diameter (frog - Barlow, 1953; mudpuppy - Karwoski and Burkhardt, 1976; tiger salamander - Wunk and Werblin, 1979 and turtle - Marchiafava, 1979). The present results are consistent with the existing literature. The cell illustrated in figure 5b displayed longer bursts of action potentials to a small spot of light (310 *um*) centered around the recording electrode than to an equiluminant stimulus that covered the whole retina. The receptive field properties of the other four stained cells were not investigated, but this finding was replicated in an unstained unit the physiological data of which are represented in figure 9. This particular unit was examined both intracellularly and extracellularly, making it highly unlikely that it was any other cell type than a ganglion cell. In the experiment portrayed, the amplitude of the EPSP evoked by a 80 *um* slit (plotted along ordinate as percent of maximal response) was obtained as a function of retinal position (plotted along the abscissa with an arbitrarily positioned zero). The largest amplitude response was obtained at four adjacent retinal locations, which allowing for slit size and stray light, describes a retinal area between 195 and 260 *um*. Although the function steeply drops off, responses approaching 35% of maximum were obtained over a 780 *um* range. Considering that the largest dendritic field of a stained ON-OFF ganglion cell was 262 *um* wide, this result emphasizes the lack of a strict relationship between the size of

dendritic and receptive fields.

ON Ganglion Cells

Figures 10 to 12 presents the data from the four ON ganglion cells successfully stained and recovered. All depolarized at light onset, their dendrites were relatively smooth and they congregated toward the vitreal half of the inner plexiform layer. Relatively few physiological experiments were performed with these cells.

The cell in figure 10b responded better to a small spot of light centered around the recording electrode than diffuse illumination, suggesting the usual center - surround antagonism. Its rectangular shaped EPSP is similar to the photic response of the depolarizing bipolar cell, which is thought to be its excitatory synaptic input (Wunk and Werblin, 1979; Frumkes, et. al., 1981). This sustained cell was also exogenously depolarized (+0.3 nA). Although this made the standing membrane potential rather unstable, transient IPSPs at light onset and offset could be observed. The upper unit (figure 10a) displayed an action potential in spite of the use of diffuse illumination; depolarizing current (+0.4 nA) failed to reveal the presence of an OFF IPSP. The remaining two units (figure 11) were only characterized by their response to diffuse illumination.

OFF Ganglion Cells

Figures 13 and 14 shows results from the two OFF ganglion cells successfully stained. Both of them appear to have dendrites that are smooth and extend deep into the inner plexiform layer. The cell of figure 12a was only characterized by diffuse illumination. Attempted analysis of the post synaptic potentials of the smaller cell (figure 12b) yielded very little information about the genesis of the hyperpolarizing response of this cell. This may be due to the opposing interactions between inhibition and disfacilitation, thereby negating the effects of current injection. Also, no difference in waveform could be detected between diffuse illumination or a slit. This is perhaps to be expected since the dendritic field is about half the size of the 80 *um* visual stimulus which may be too gross for adequate testing.

Amacrine Cells

General Anatomical Properties

Figures 15-27 present data from the amacrine cells intracellularly stained with HRP. This designation was made, since after careful scrutiny, all of these cells were found to be without a visible axon. Most of the amacrine cells encountered (70%) had their soma's conventionally placed in the inner nuclear layer. As intracellular staining studies in mammals have shown (Bloomfield, 1981; Nelson, 1982), some of the units were observed to have their somas "displaced" to the ganglion cell layer.

The thicker dendrites (approximately 2 *um*) of some amacrine cells project a considerable distance from the soma, with deliberate, angular branching of secondary and tertiary dendrites (figures 16, 17a, 21d, and 26a). In general, the amacrine cells that appear more diffusely organized have many of the thinner dendrites emanating from the perikarya, which then profusely divide and fill the dendritic field (figures 19 and 26b). The dendrites of some amacrine cells have varicosities and support various appendages such as stalks and spines. These are not apparent on the dendrites of the ganglion cells just described.

ON-OFF Amacrine Cells

Figures 15 and 16 show the stereotypical large and transient depolarizing photic responses of two examples of ON-OFF amacrine cells. Cells that displayed spikes under similar stimulus conditions (diffuse light) were also recovered (figures 17a & b). The cell of figure 17a has intracellular responses that were recorded before (upper) and after (lower) staining. Multiple spikes were seen in both records, where the upper record displayed small, transient IPSPs and the lower record displayed the stereotypical EPSPs. It is quite possible that these different observations reflect the amount of injury incurred by electrode penetration, and/or the capricious influence of HRP injection. Most cells were unaffected by the HRP injection; however sometimes even without stain, the wave form of a cell can be changed due to an improved seal between the membrane and the electrode or injury (Miller, 1979). The accompanying photomicrograph (figure 18), of the same unit, shows that the unit was cleanly stained indicating it unlikely that the electrode moved into another cell. In any event, there was no obvious correlation between these different response patterns and morphology.

The cell shown in figure 19 is an interesting case for two reasons. First, although the soma of this cell was lo-

cated in the inner nuclear layer, one neurite was found to leave the general dendritic field. Initially this neurite was considered to be an axon, forcing one to conclude that this cell is a displaced ganglion cell. However, this neurite did not take a straight route toward the optic disk. Instead, from its origin, the branch of a dendrite, it went past the middle of the retina and continued toward the opposite edge, over 500 *um*. This process remained within the inner plexiform layer and an occasional protrusion of a stalk or spine could be seen. Other cells have been observed that will project 1 or 2 dendrites, that can be traced over 300 *um* and do not approach the optic disk. The second notable feature of this cell is that its intracellularly recorded photic response displayed prominent IPSPs without any signs of excitation (record not illustrated), in a manner similar to the cell of figure 17a (upper record). Due to the unusual anatomical and physiological characteristics of this cell, determining whether or not it is an amacrine or ganglion cell is difficult. Although it shares properties of both, it is included with the amacrines because none of its processes appear to leave the retina. Inhibition in amacrine cells is not usually observed, however, this is perhaps due to the fact there are so few intracellularly stained examples. The presence of IPSPs in amacrine cells would be a sign of interactions within this class of inhibitory neurons and congruent with the complex synaptic arrangements observed.

The receptive fields of ON-OFF amacrine cells are fairly large and uniform when tested with concentric stationary spots (Werblin and Copenhagen, 1974). Figure 20 shows similar results that were obtained from the determination of the receptive field from an unstained amacrine cell. Verification of this unit as an amacrine cell was by physiological and pharmacological criteria, such as: depth of penetration, EPSPs virtually the same as the two ON-OFF amacrine cells of figures 15 & 16 (not illustrated). Furthermore, GABA and glycine eliminated the light evoked response, both substances had little influence on the dark potential of the cell (Miller, et al., 1981b). This cell gave the same response to a 80 *um* slit as to diffuse illumination. When the slit was stepped across the receptive field (in a manner identical to the ON-OFF ganglion cell of figure 14) for 780 *um* the amplitude of the EPSPs did not even reach the half maximal point.

ON Amacrine Cells

Figures 21-25 represents data from the successfully stained ON amacrine cells. The present sample is divided into two groups. One group (figures 21 & 22) has their cell bodies conventionally located in the inner nuclear layer. The second group (figures 23 to 25) has their cell bodies displaced within the ganglion cell layer. This later group

of cells are considered to be displaced amacrine cells for three reasons. First, the most careful observation fails to reveal an axon. In contrast, many ganglion cells, even if only lightly stained, will display their axons. Second, their responses appear very similar to the ON cells whose perikarya are in the inner nuclear layer: a sustained EPSP with transient IPSPs. Depolarization, whether cause by current injection (figure 21b) or injury (figure 21d), revealed transient OFF IPSPs for cells with their perikarya in the inner nuclear layer. Depolarizing current also enhanced the presence of IPSPs in the displaced units. Finally, the receptive field of these cells can be demonstrated to be very similar to ON-OFF amacrine cells.

Conventionally placed ON amacrines have rather modest dendritic fields. The dendrites of the top two cells of figure 21 (a & b) have many varicosities. The lower 2 cells' dendrites (c & d) had somewhat larger dendritic fields consisting of processes with few irregularities. The displaced ON amacrines had somewhat fuller dendritic fields and no special appendages were found. The receptive fields of both types of ON amacrines are large and homogeneous. The response to a 80 um slit was either the same or slightly smaller than to diffuse illumination and no evidence of center - surround antagonism could be found. The lateral extent of the receptive field was determined in several ON cells by stepping a slit across the retina in a manner previously described for the ON-OFF units. Figure 25 describes

results obtained with a displaced amacrine cell (23a) and an unstained ON cell, to be further described (figure 35). Like the transient unit previously described (figure 20), the attenuation in amplitude of the EPSP of these cells exceeds the half maximal amplitude recorded over a 700-800 *um* wide area. Assuming the unstained unit is morphologically similar to the others, these results again describe the apparent lack of correlation between dendritic and receptive field geometries.

OFF Amacrine Cells

Figures 26 and 27 show stained examples of OFF amacrine cells and to my knowledge, these are the first to be morphologically identified in the mudpuppy retina. Because so few OFF units were encountered, the primary concern was to successfully stain the cells; therefore physiological observations of these cells were very limited. There is an interesting, but speculative, correspondence between the amplitude of the graded potentials and the thickness of the dendrites. The cell of figure 26a had dendrites that were relatively thick (1 to 2 *um*) and its photic response was dominated by a prominent and sustained hyperpolarization. In contrast, the cell of figure 26b had varicose dendrites which were rather thin (< 0.5 *um*) and displayed only a small transient IPSP at light offset, in which current in-

jection was unable to exaggerate any graded potentials at light onset.

One hypothesis concerning the inability of current injection to affect the PSPs of some OFF cells, as was previously mentioned, is the cancellation effect between opposing PSPs. Another possiblilty, evidenced by the difference in the sustained potentials, is the low pass cable charateristics of neural processes of wide diameter. Electrotonic spread of current is facilitated by processes with the large time and length constants accompanying thicker dendrites (Junge, 1981). Therefore sustained application of current should also be less effective in cells with finer processes. This latter hypothesis would therefore predict the observed differences in the photic responses of these two different shaped units.

TABLE 1: Anatomical measurements of cells categorized by their excitatory potentials.

A	B	C	D	E	F	G	H	I
ON-OFF Ganglion cells.								
5a	20.0	22.5	232	262	5.0	22.0	13.5	6.5
5b	16.0	18.5	125	250	6.0	18.0	12.0	8.0
6a	17.5	22.0	113	225	7.0	21.0	14.0	6.0
6b	19.0	27.0	112	250	4.5	17.5	11.0	9.0
6c	12.5	15.0	65	75	5.0	13.0	9.0	11.0
AVG	17.0	21.0	130	212	5.5	18.3	11.9	8.1
ON Ganglion cells								
10a	12.5	32.0	115	147	4.0	10.0	7.0	13.0
10b	20.0	20.5	130	360	2.0	10.0	6.0	14.0
11a	12.5	12.5	200	200	4.0	9.0	6.5	13.5
11b	11.0	17.5	68	200	3.5	9.5	6.5	13.5
NI	14.4	17.5	137	137	5.0	12.0	8.5	11.5
Displaced ON Amacrine cells								
23a	10.2	16.6	88	152	4.0	11.0	7.5	12.3
23b	15.0	20.0	113	170	3.5	10.5	7.0	13.0
23c	15.3	18.0	108	188	2.0	7.0	4.5	15.5
AVG	13.9	19.3	120	194	3.5	9.9	6.7	13.3
OFF Ganglion cells								
13a	17.0	21.0	275	325	13.0	23.0	18.0	2.0
13b	14.0	15.0	45	50	6.0	15.0	10.5	9.5
AVG	15.5	18.0	160	187	9.5	19.0	14.3	5.8
ON-OFF Amacrine cells								
15	17.5	20.0	365	365	4.0	18.0	11.0	
16	10.0	17.5	150	325	3.0	15.0	9.0	
17a	20.0	22.5	250	250	4.0	21.0	12.5	
17b	14.0	16.0	87	130	6.0	22.0	14.0	
NI	14.0	15.0	90	130	4.0	17.0	10.5	
AVG	15.1	18.2	188	240	4.2	18.6	11.4	
ON Amacrine cells								
21a	17.0	17.0	83	117	4.5	12.5	8.5	
21b	11.4	14.0	45	70	10.0	20.0	15.0	
21c	15.0	15.0	75	200	12.0	23.0	17.5	
21d	12.5	20.0	87	162	7.0	21.0	14.0	
AVG	14.0	16.5	73	137	8.4	19.1	13.8	
OFF Amacrine cells								
26a	11.5	19.1	325	400	4.0	9.0	6.5	
26b	9.0	16.0	162	200	2.5	7.5	5.0	
AVG	10.2	17.6	243	300	3.3	8.3	5.8	

See next page for legend.

A Figure in which camera lucida drawing of cell appears.
B Cell body width (along minor axis) in *um*
C Cell body width (along major axis) "
D Dendritic field (along minor axis) "
E Dendritic field (along major axis) "
F Distance from cell body (point of best focus) to closest dendrite seen, measured in *um*
G Distance from cell body (point of best focus) to farthest dendrite seen, measured in *um*
H Estimated middle of dendritic field, (F+G)/2
I Projection into inner plexiform layer by ganglion cells (20-H).
Correction not needed for amacrine cells.
Therefore the projection is same as H.

All measurements represent an average of at least three observations.

NI- not illustrated.

Figure 3. Light micrograph of the mudpuppy retina in a 1 *um* thick radial section showing the nuclear and plexiform layers. OS, outer segments; IS, inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; INL, inner plexiform layer; GCL, ganglion cell layer. X 1600.

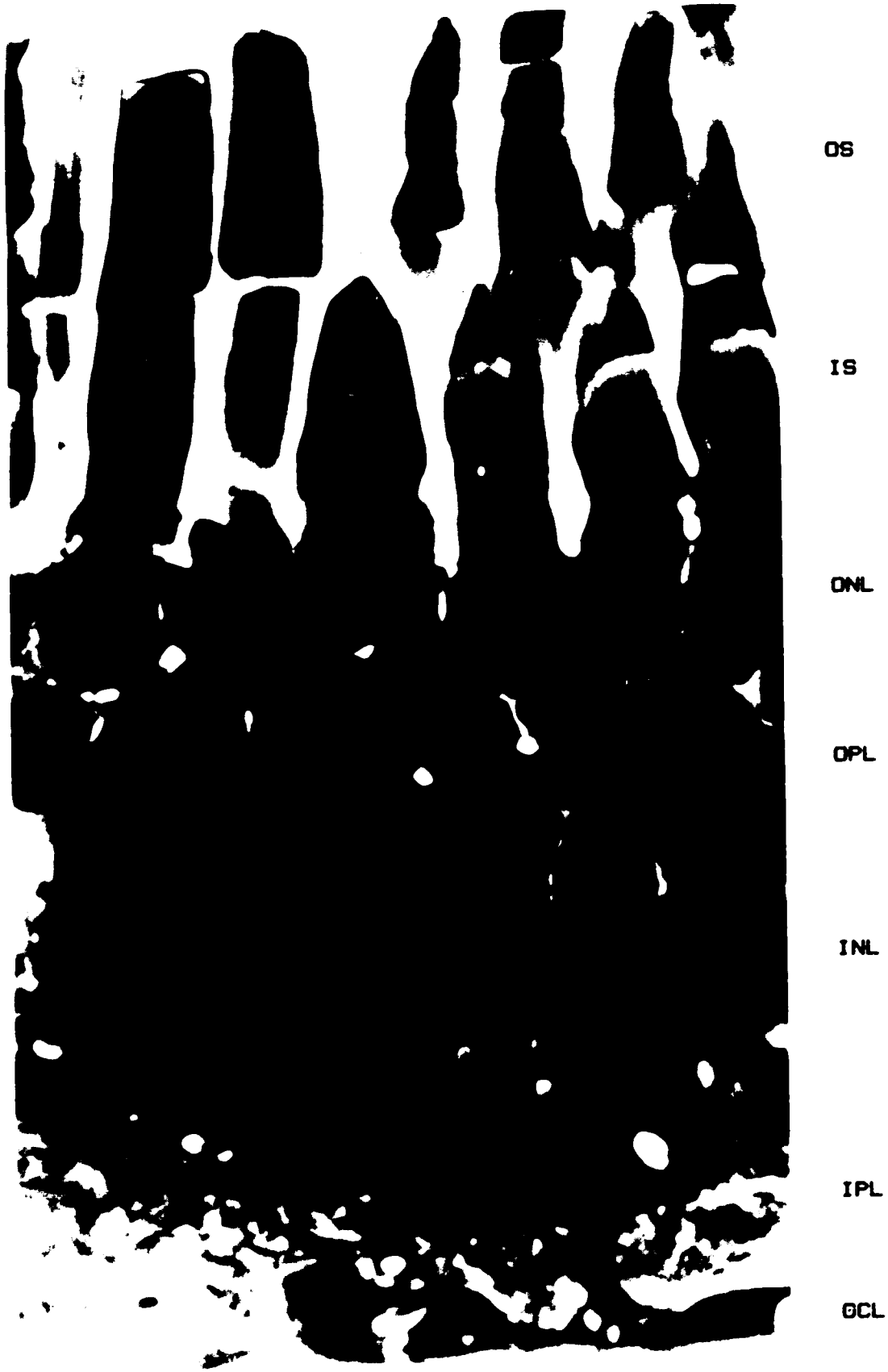

OS
IS
ONL
OPL
INL
IPL
GCL

Figure 4. Light micrograph of a quadrant of flat mounted mudpuppy retina, stained by retrograde transport of Horseradish peroxidase. The retina is seen from its vitreal surface and shows the collections of ganglion cell axons forming into thick trunks, projecting to the optic disk in the center of the eye (lower left corner). X 430.

Figure 5. Camera lucida drawings of two ON-OFF ganglion cells. The illustrated intracellular response from the top cell (a) is to a 2 sec duration, diffuse light flash. When this cell was depolarized from its resting potential of -57 mV with +0.5 nA, transient ON-OFF IPSPs were observed (not shown). For the cell on the bottom (b), the illustrated responses are to either a 310 *um* spot or diffuse illumination, indicated respectively by the large and small spots. For these and all other illustrations, spike height was attenuated by the response characteristics of the polygraph, but no attempts were made to extend them by retouching the photographs. Axons indicated by arrows.

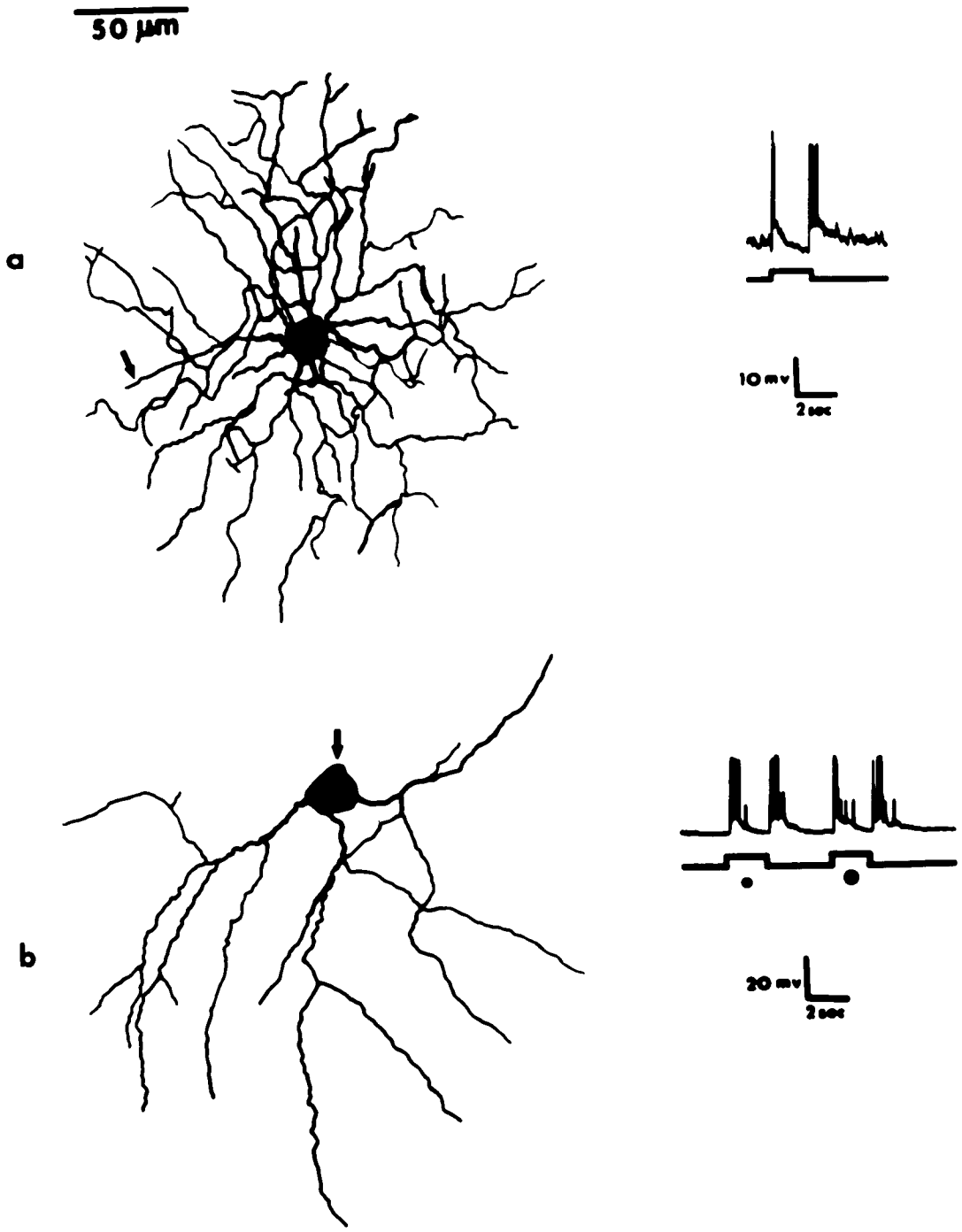

50 µm
a
10 mv
2 sec
b
20 mv
2 sec

Figure 6. Camera lucida drawings of three ON-OFF ganglion cells. The middle Cell (b) had a resting potential of -52 mV. The bottom cell (c) spontaneously displayed its IPSPs (resting potential -42 mV), which were blocked by 10 uM of picrotoxin. No difference could be detected in the response to diffuse illumination and a 100 um slit. Axons indicated by arrows.

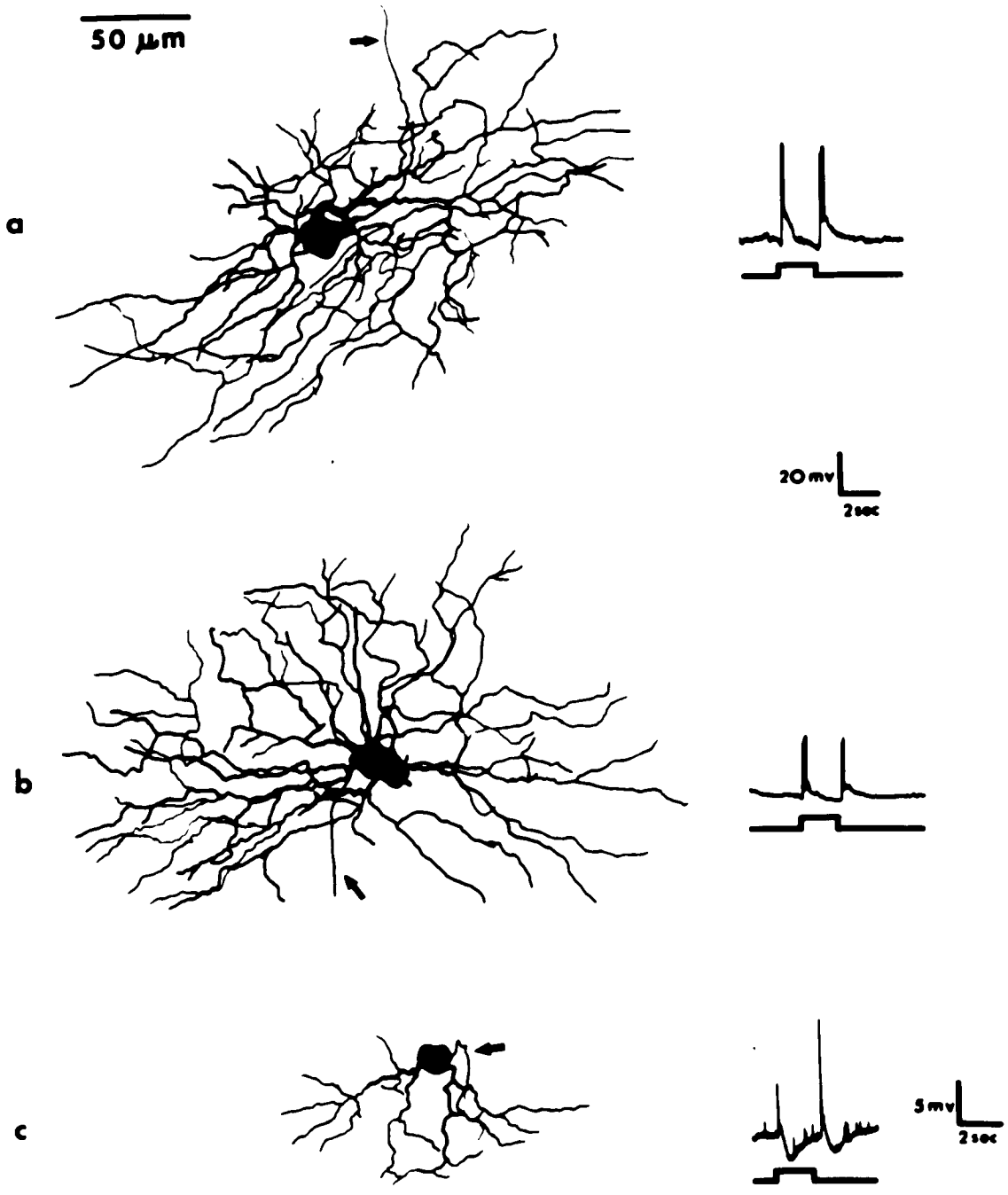

50 µm
a
20 mv
2 sec
b
c
5 mv
2 sec

Figure 7. Photomicrograph of the flat mounted, ON-OFF ganglion cell illustrated in Figure 5a. X 956.

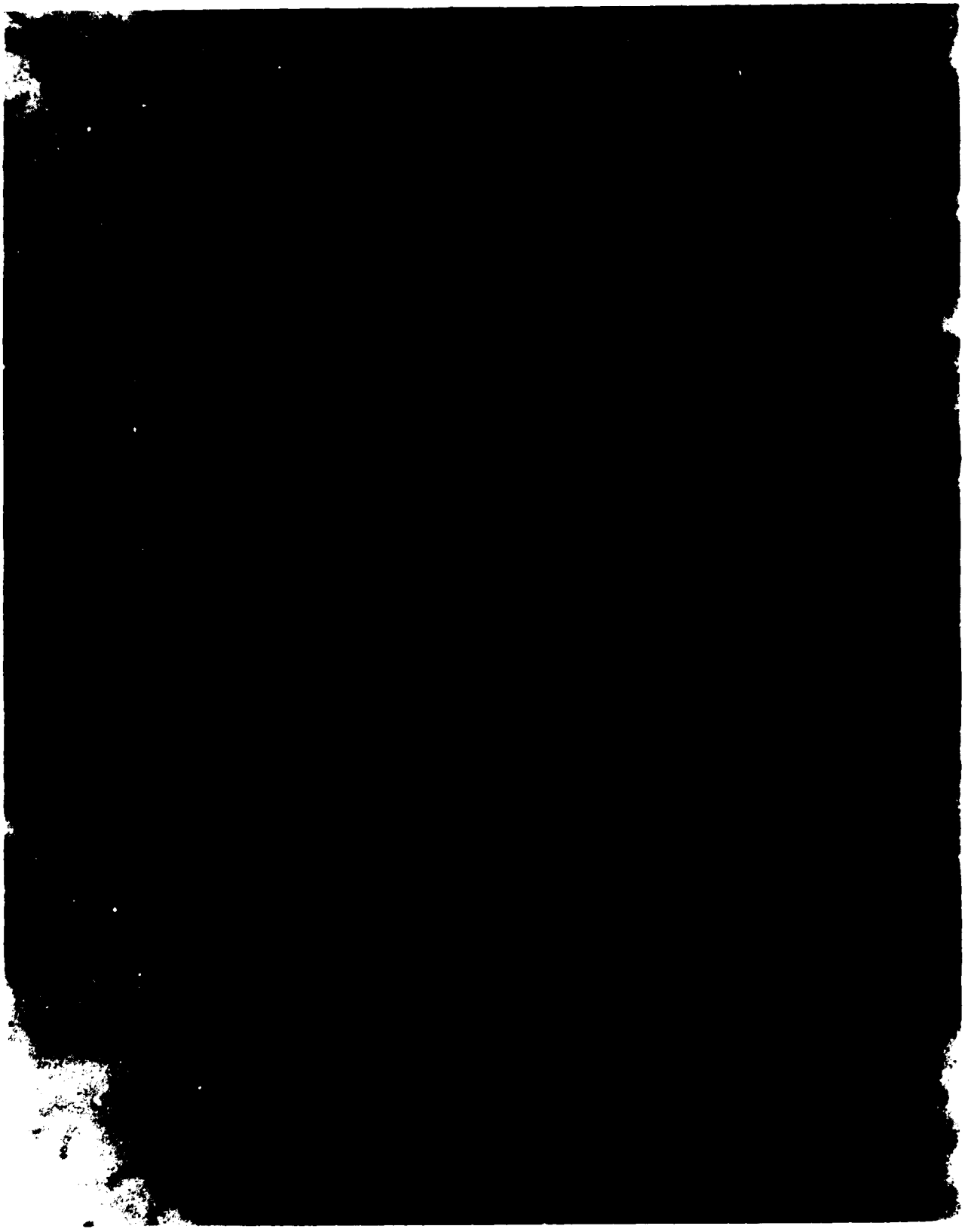


Figure 8. Photomicrograph of a ganglion cell that only demonstrated transient ON-OFF IPSPs. X 875.

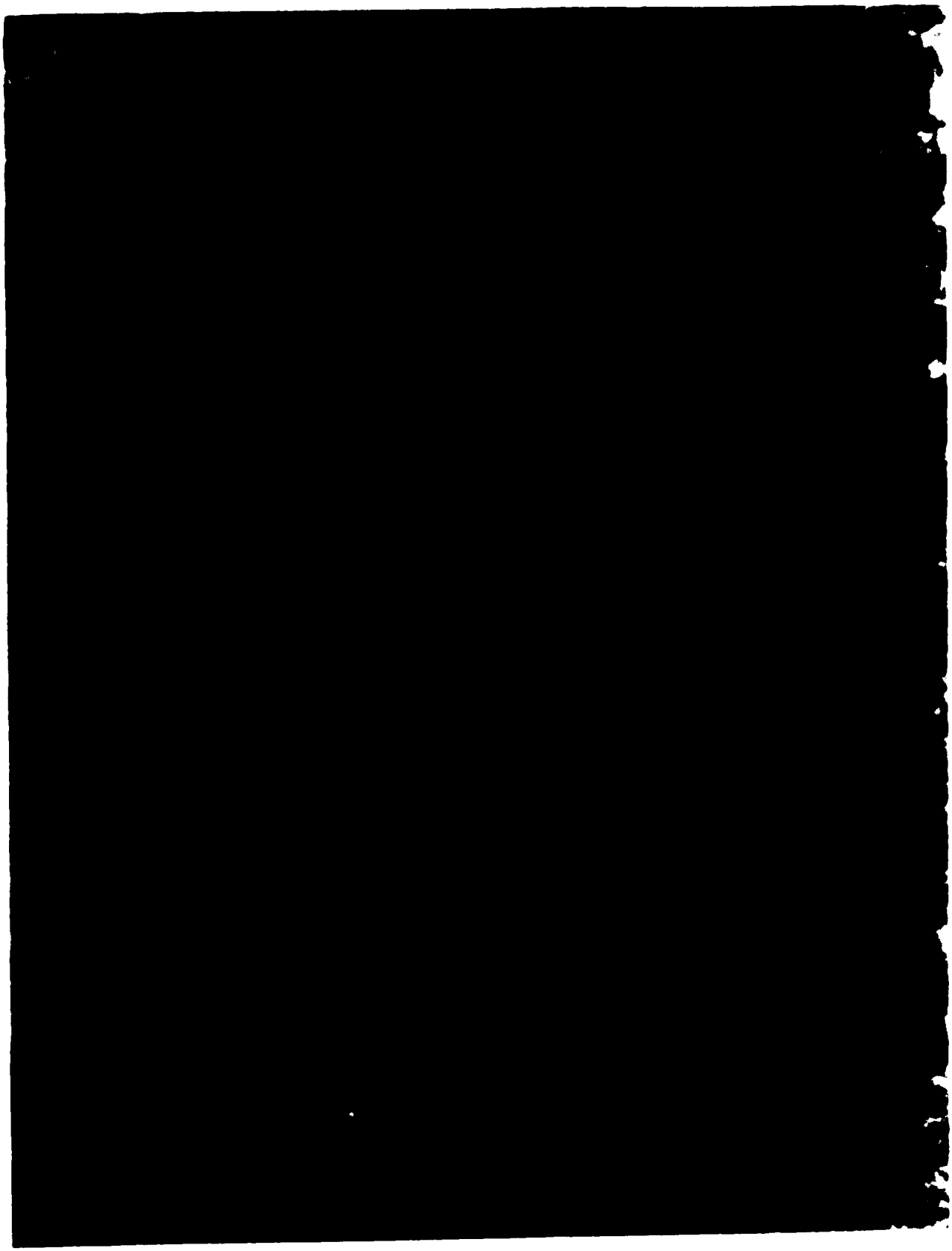


Figure 9. Receptive field of an unstained ON-OFF ganglion cell. The amplitude of the EPSP (along the ordinate as % of maximum) evoked by an 80 *um* slit is plotted as a function of slit position (along abscissa in *um*, with arbitrary zero position). Both intracellular and extracellular records were obtained from this cell (not illustrated). The resting potential of the cell was -68 mV and no IPSPs were observed.

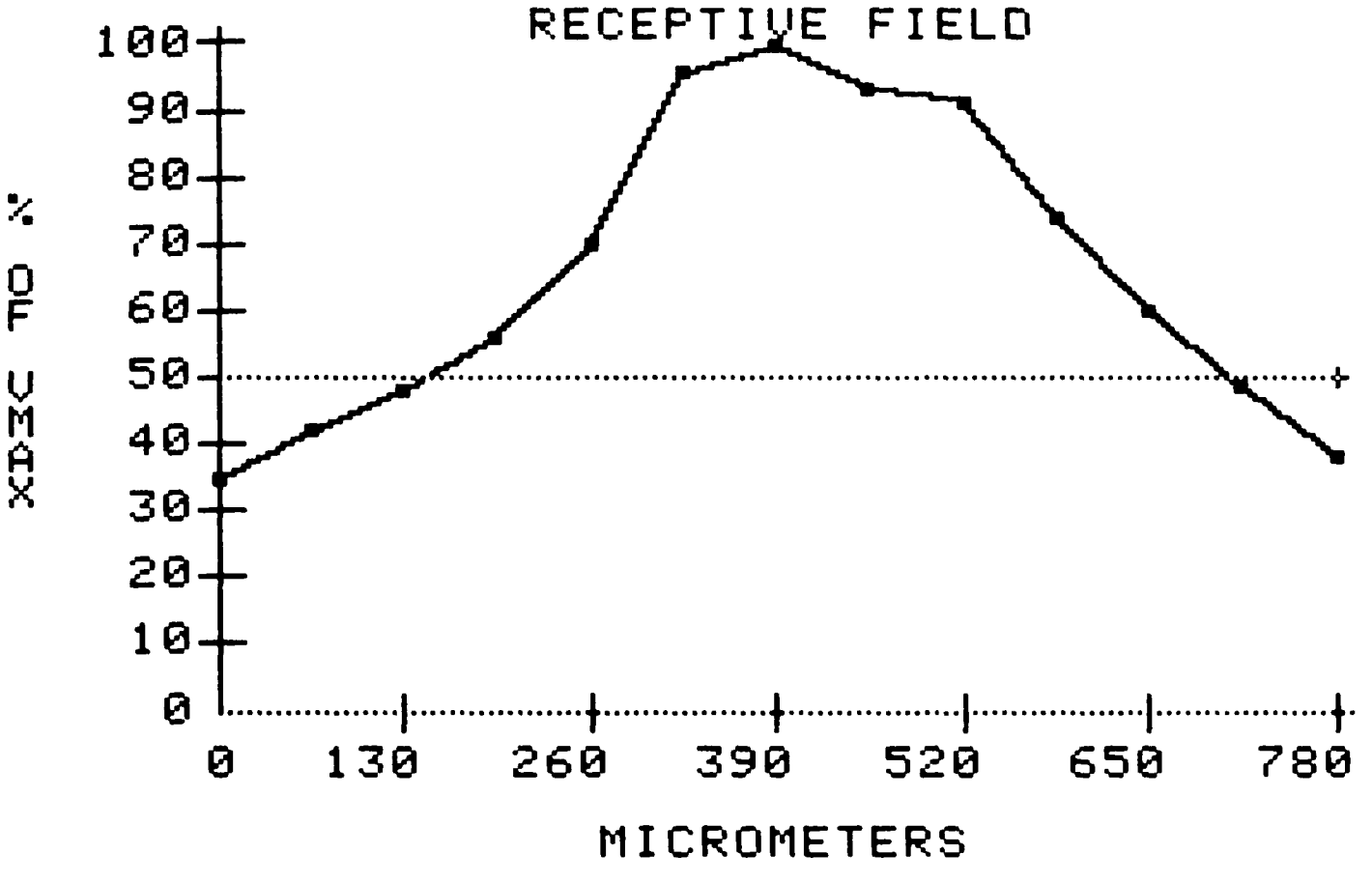

RECEPTIVE FIELD
% OF MAX
100
90
80
70
60
50
40
30
20
10
0
0
130
260
390
520
650
780
MICROMETERS

Figure 10. Camera lucida drawings of two ON ganglion cells. The photic response of the upper cell (a) consisted of a transient spike at light onset followed by an IPSP. The response of the lower cell (b) to a 510 *um* spot, was characterized by sustained spiking and EPSP. Diffuse illumination produced only a small response. Axons indicated by arrows.

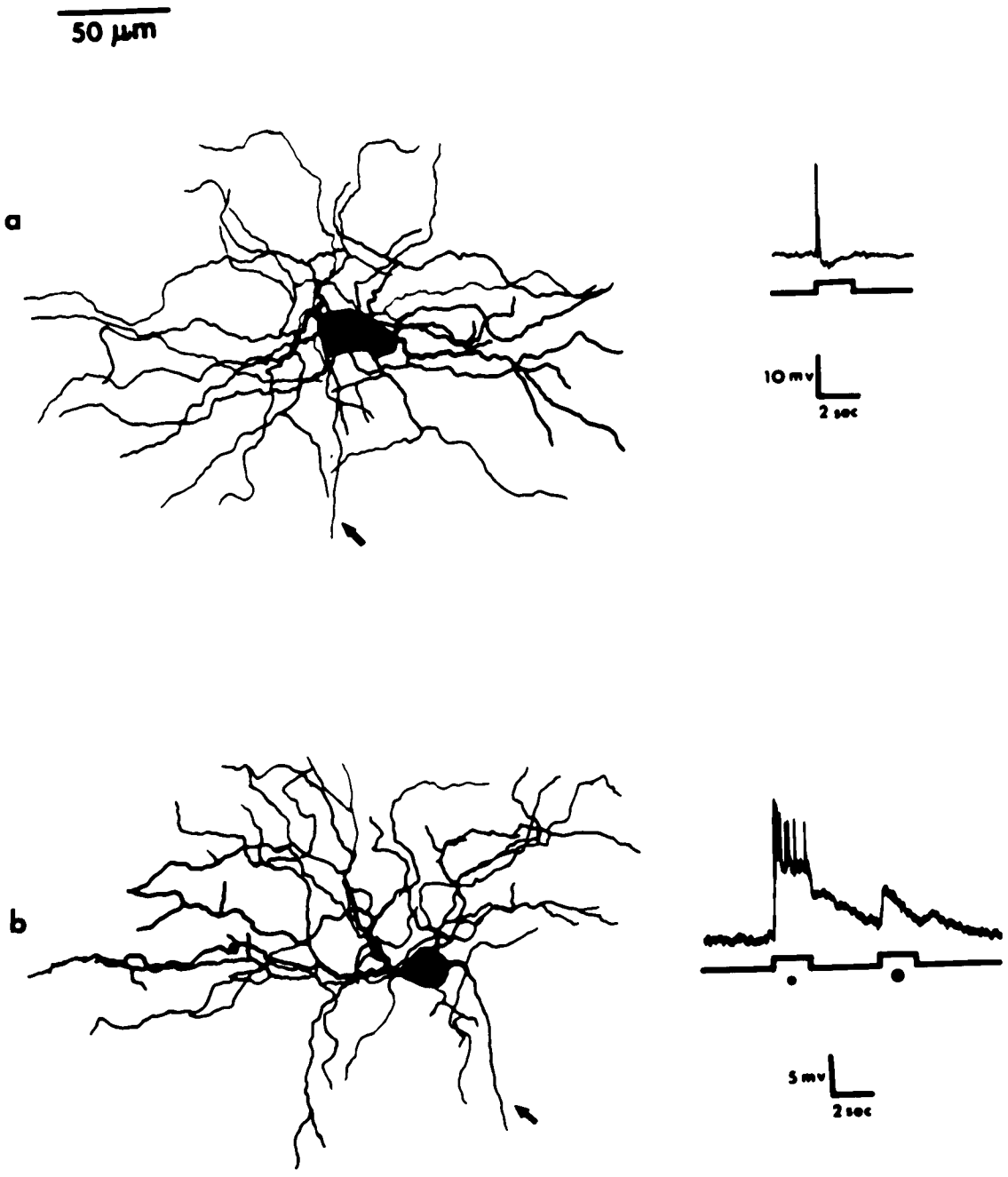

50 µm
a
10 mv
2 sec
b
5 mv
2 sec

Figure 11. Camera lucida drawings of two ON ganglion cells. The axon of the upper cell (a) could not be found due to artifactual, extracellular staining that surrounded the soma. Therefore classification of this unit as a displaced amacrine cell would be precarious. The lower cell (b) rapidly depolarized after impalement of the electrode and disabled the cell's spiking mechanism. Axons indicated by arrows.

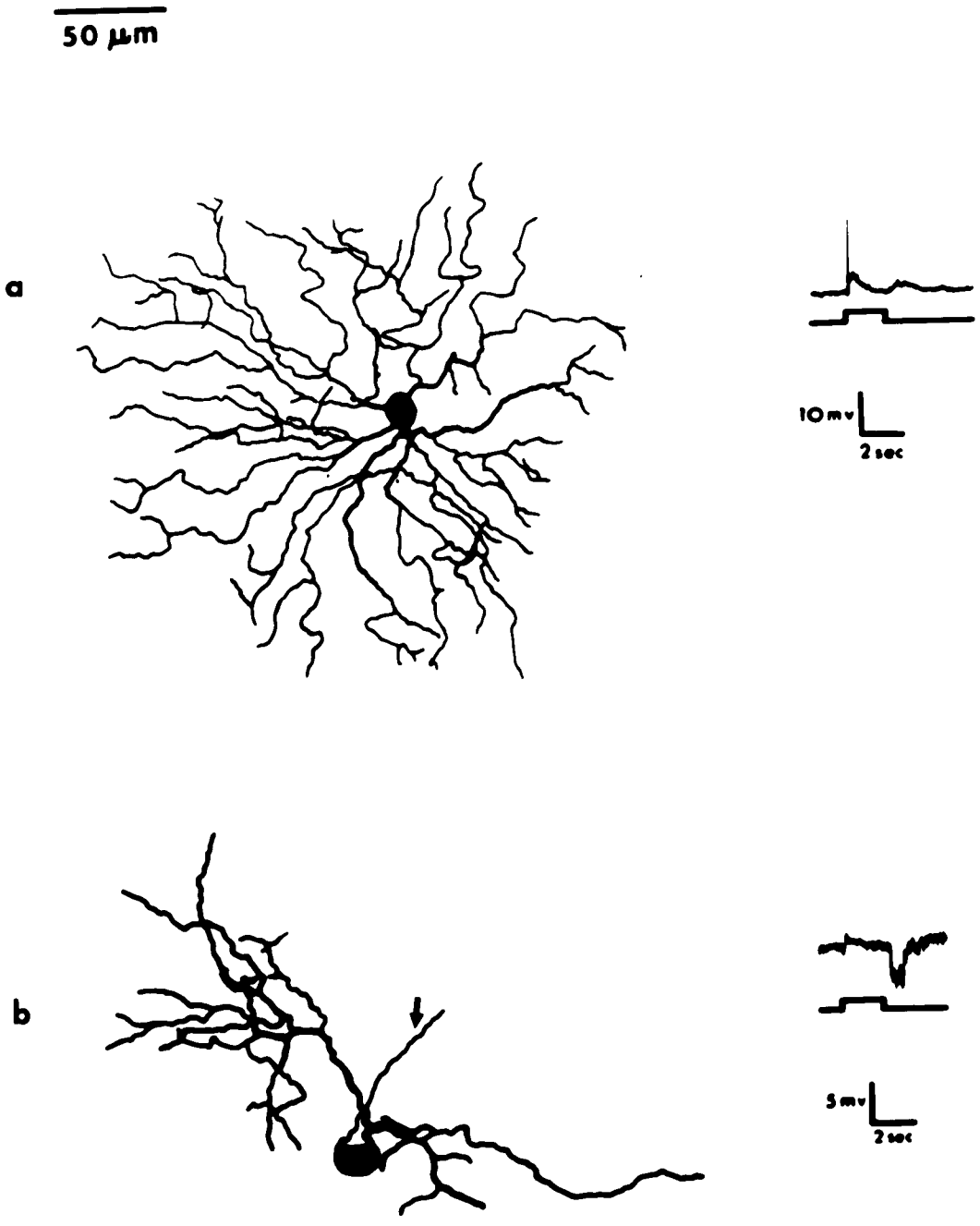

50 µm
a
10 mv
2 sec
b
5 mv
2 sec

Figure 12. Photomicrograph of the flat mounted, ON ganglion cell illustrated in Figure 10a. X 875.

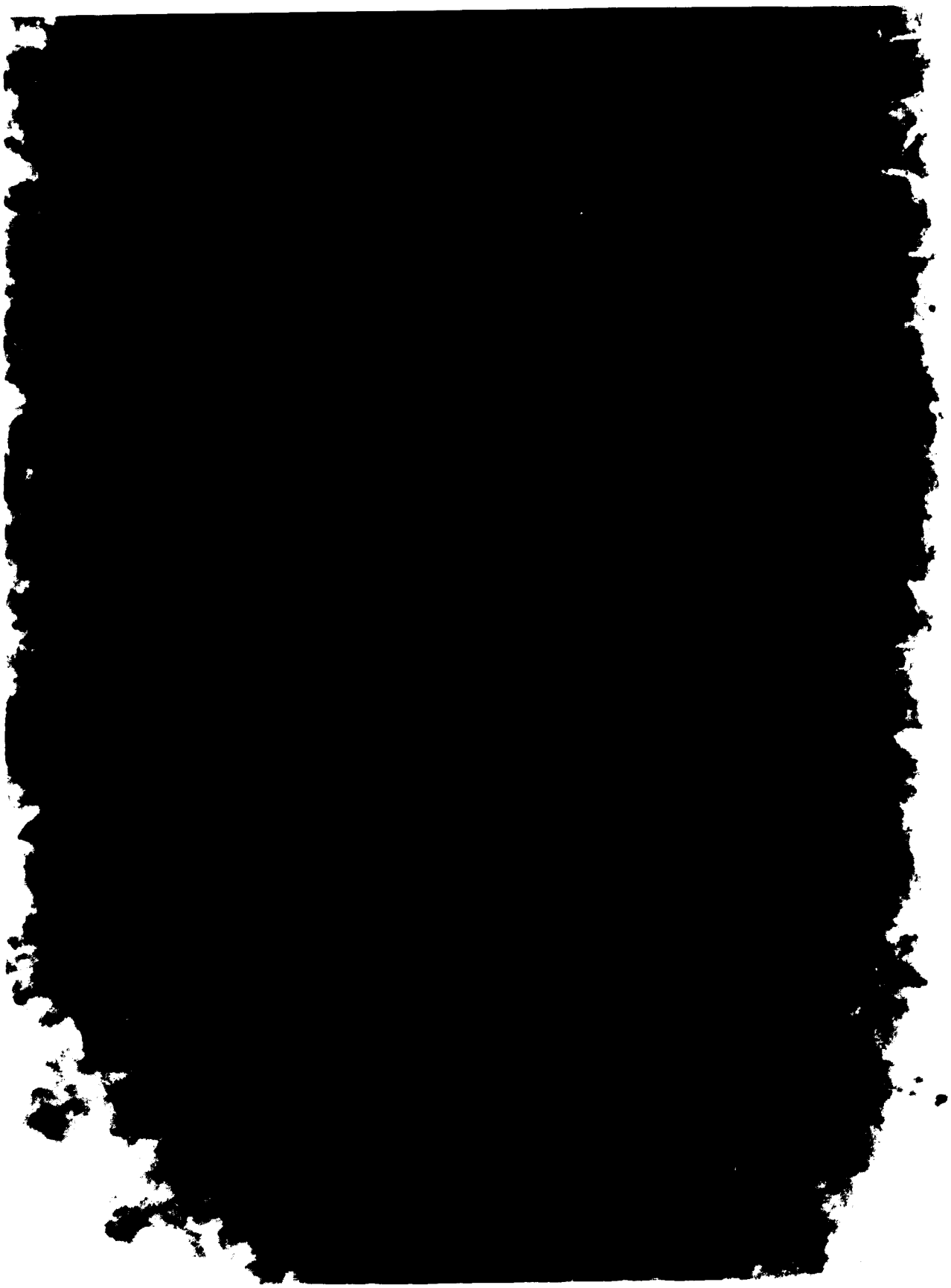


Figure 13. Camera lucida drawings and intracellular responses of two OFF ganglion cells. Axons indicated by arrows.

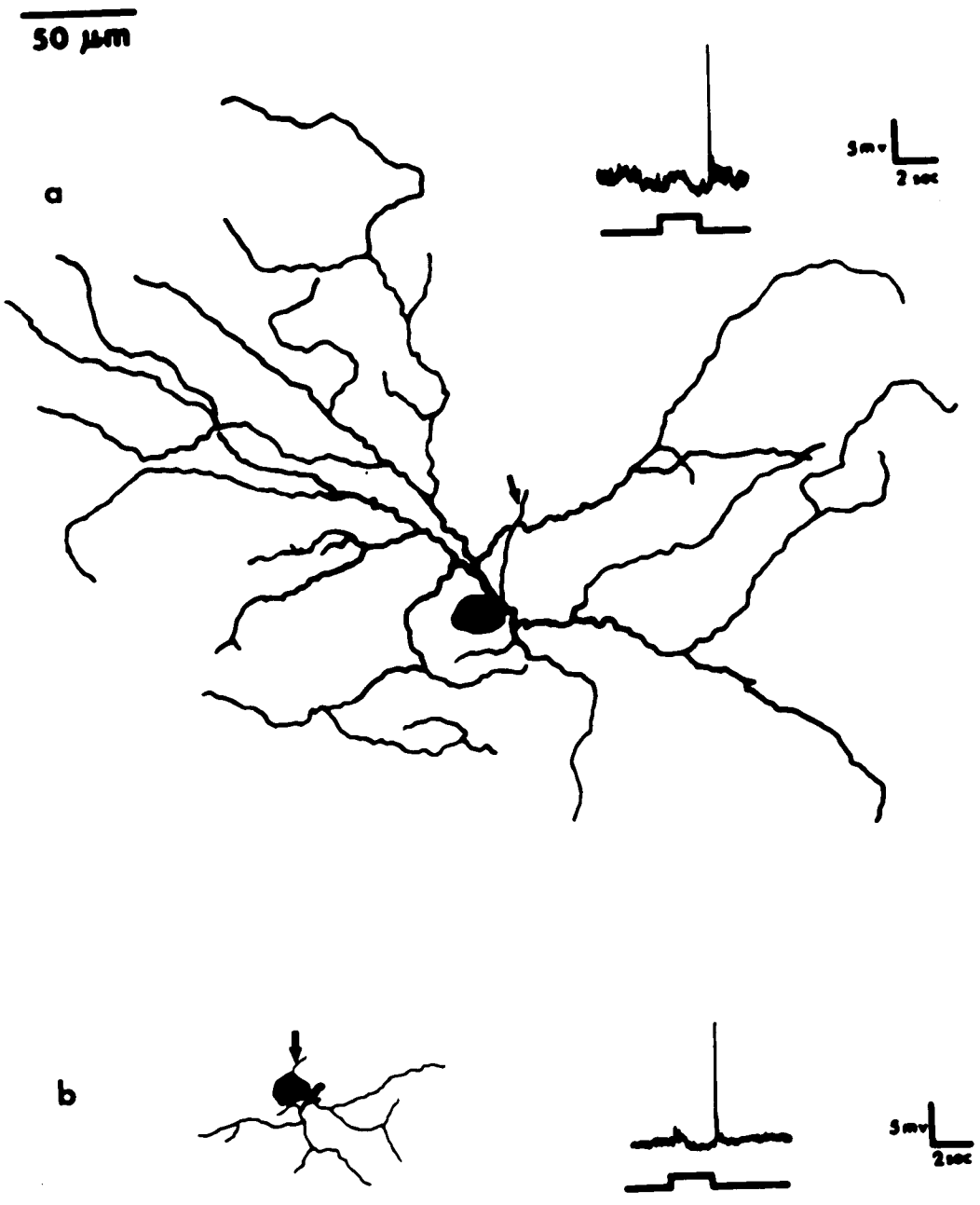

50 µm
a
5mv
2 sec
b
5mv
2sec

Figure 14. Photomicrograph of the flat mounted, OFF ganglion cell illustrated in Figure 13b. X 2000.

Figure 15. Camera lucida drawing and intracellular response of an ON-OFF amacrine cell. When viewed under high power, the neurites of this cell could be seen to occasionally support varicosities and specialized appendages such as stalks and spines.

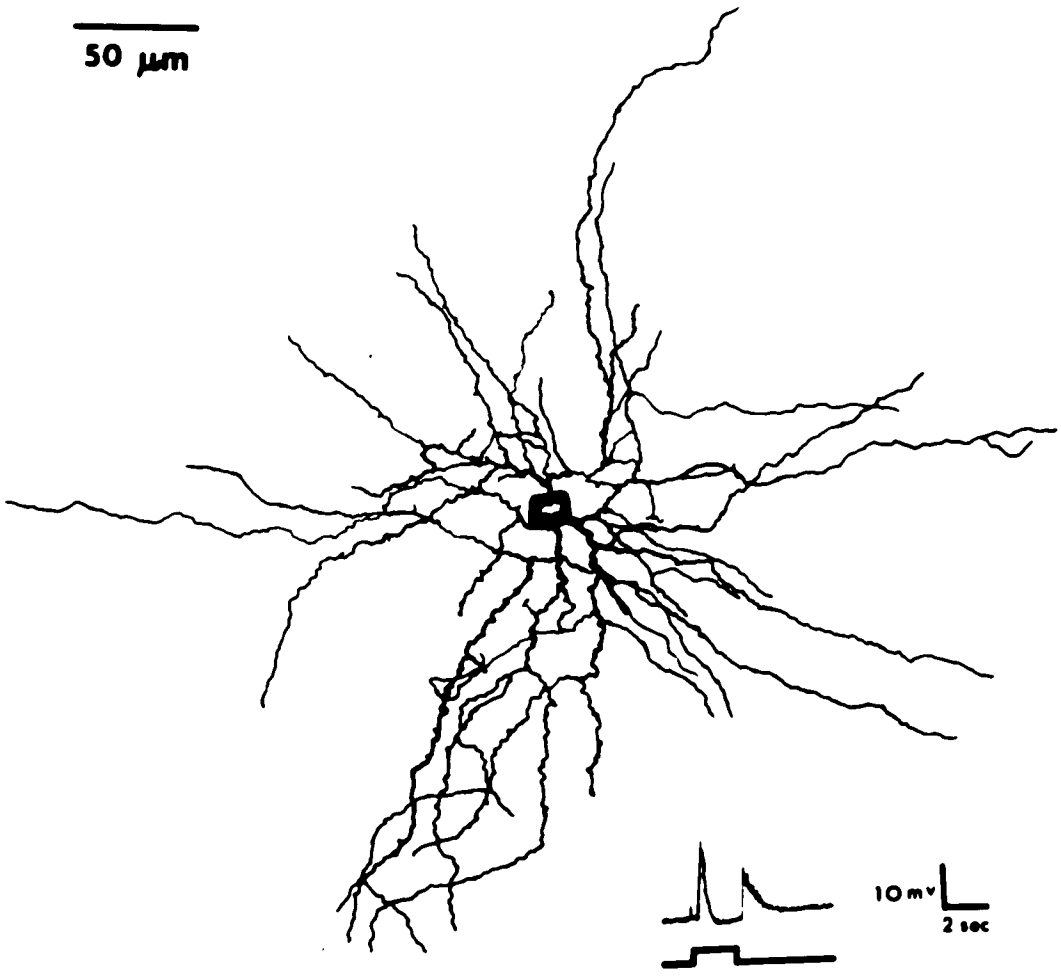

50 µm
10 mv
2 sec

Figure 16. Camera lucida drawing and intracellular response of an ON-OFF amacrine cell. The neurites of this cell appeared rather uniform.

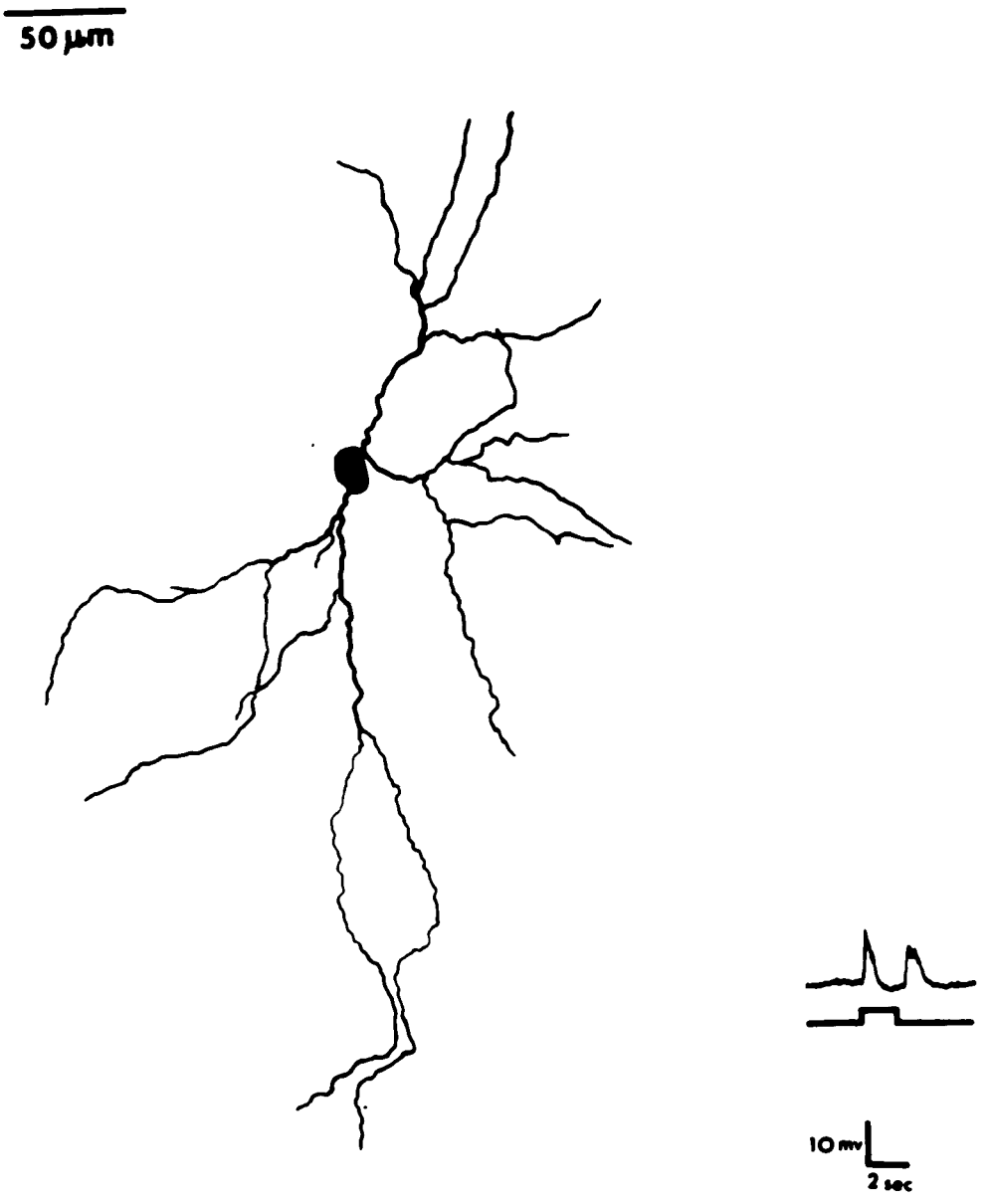

50 µm
10 mv
2 sec

Figure 17. Camera lucida drawings and intracellular responses of two ON-OFF amacrine cells. Two samples of the intracellular record are provided for the top cell (a) before (upper) and after (lower) staining. See text for details.

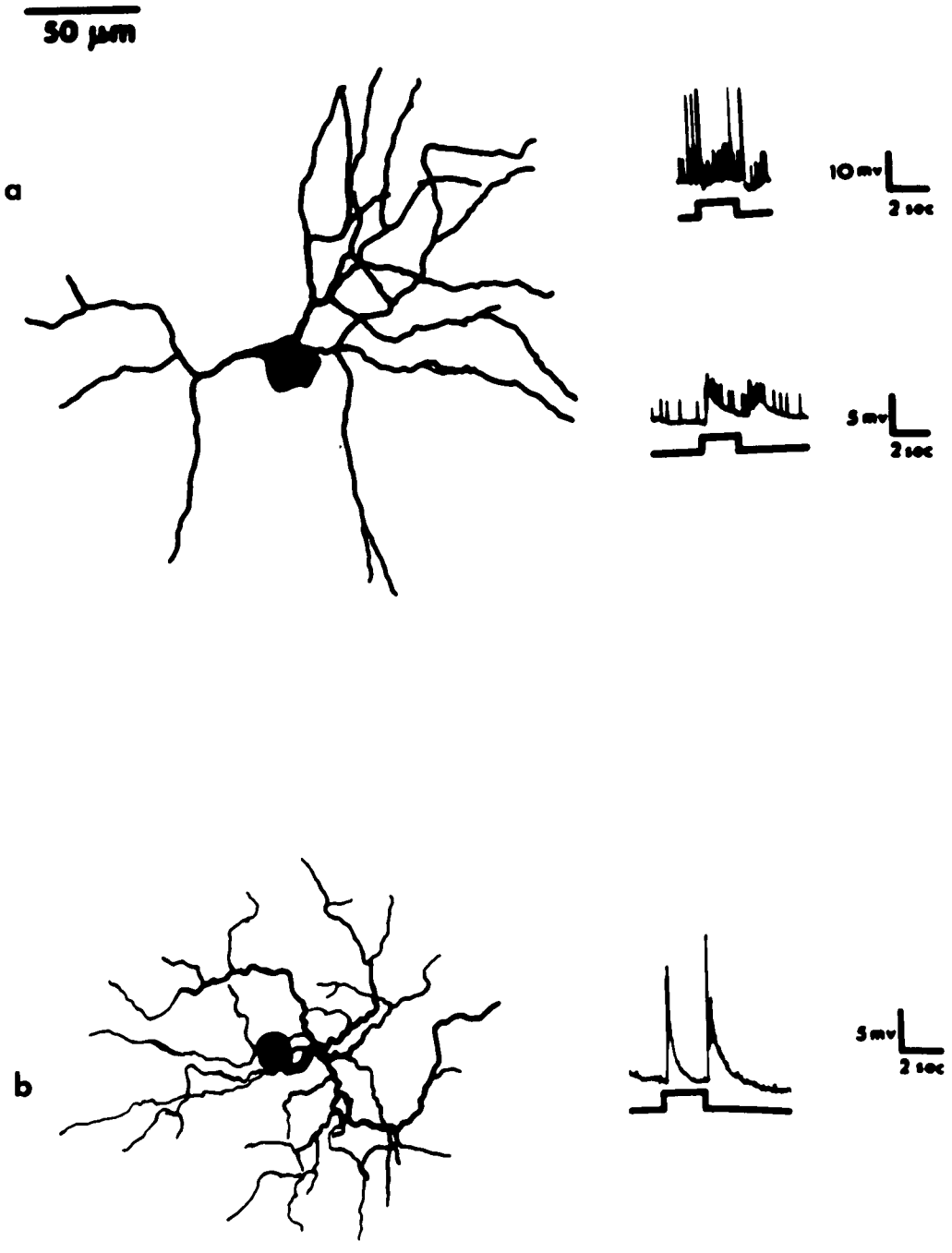

50 µm
a
10 mv
2 sec
5 mv
2 sec
b
5 mv
2 sec

Figure 18. Photomicrograph of the flat mounted, ON-OFF amacrine cell illustrated in Figure 17a. X 956.

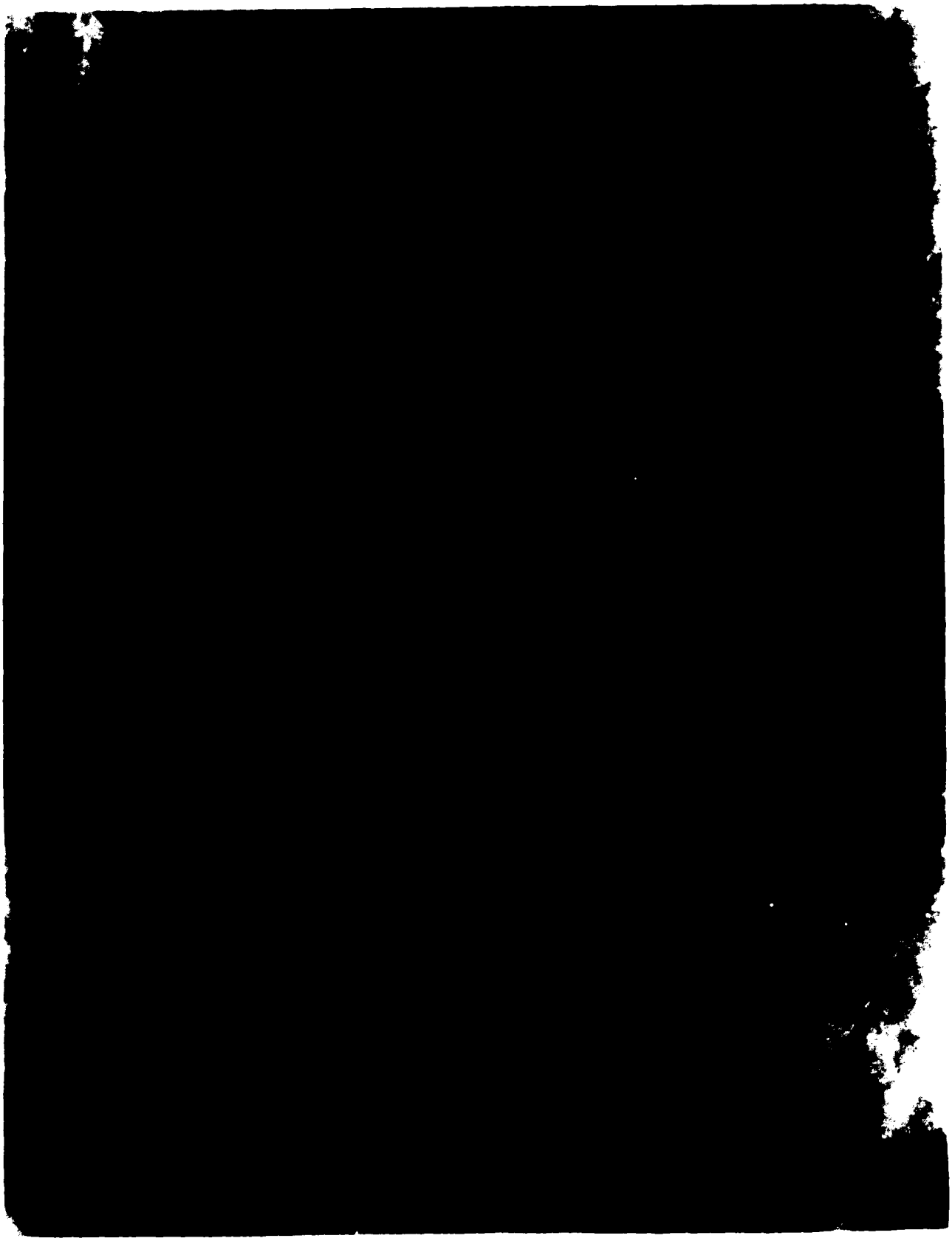


Figure 19. Photomicrograph of a flat mounted, amacrine cell. This cell's intracellular record displayed ON-OFF IPSPs. See text. X 736.

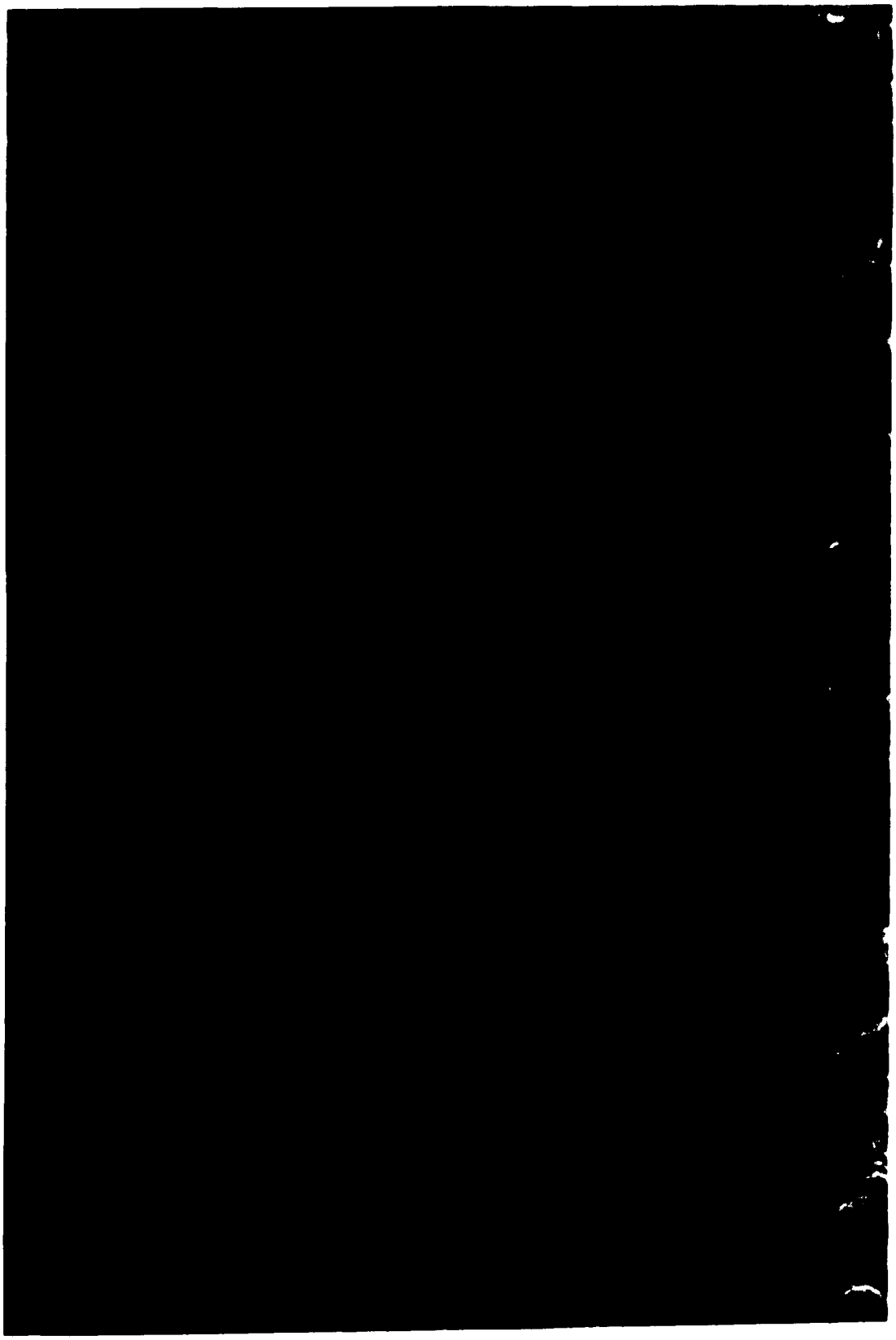


Figure 20. Receptive field of an unstained ON-OFF amacrine cell. EPSP amplitude (along the ordinate as % of maximum) evoked by an 80 *um* slit is plotted as a function of slit position (along abscissa in *um*, with arbitrary zero position). See text and legend of figure 9 for further explanation. Introduction of 2 mM GABA caused the loss of light evoked activity in this neurons, but did not hyperpolarize it. The effects of picrotoxin and strychnine (20 *u*M) were to slightly enhance the amplitude of the spikeless EPSPs.

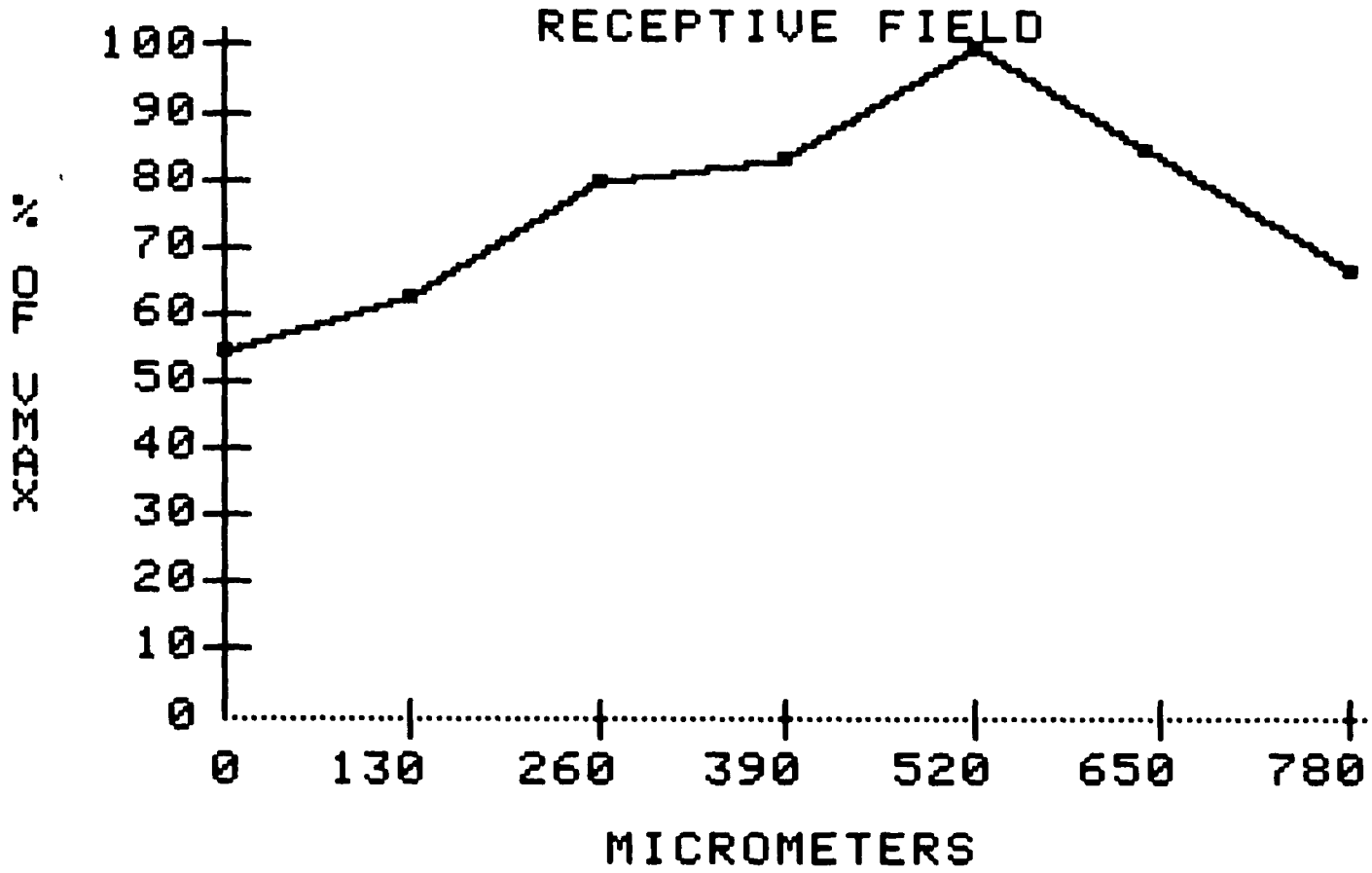

RECEPTIVE FIELD
% OF VMAX
100
90
80
70
60
50
40
30
20
10
0
0
130
260
390
520
650
780
MICROMETERS

Figure 21. Camera lucida drawings and intracellular responses of 4 ON amacrine cells with conventionally located perikarya in the inner nuclear layer. The dendrites of the first cell (a) had many varicosities and had a beaded appearance like dew on a spider's web. The second cell (b) also had numerous varicosities, however they could be better described as bulges that occur along the length of the dendrite. The dendrites of the bottom 2 cells (c & d) were generally without any specialized appendages, however there were occasional bulges in the dendrites observed under high power. The photic response of the bottom cell (d) is to diffuse illumination and an 80 μm slit.

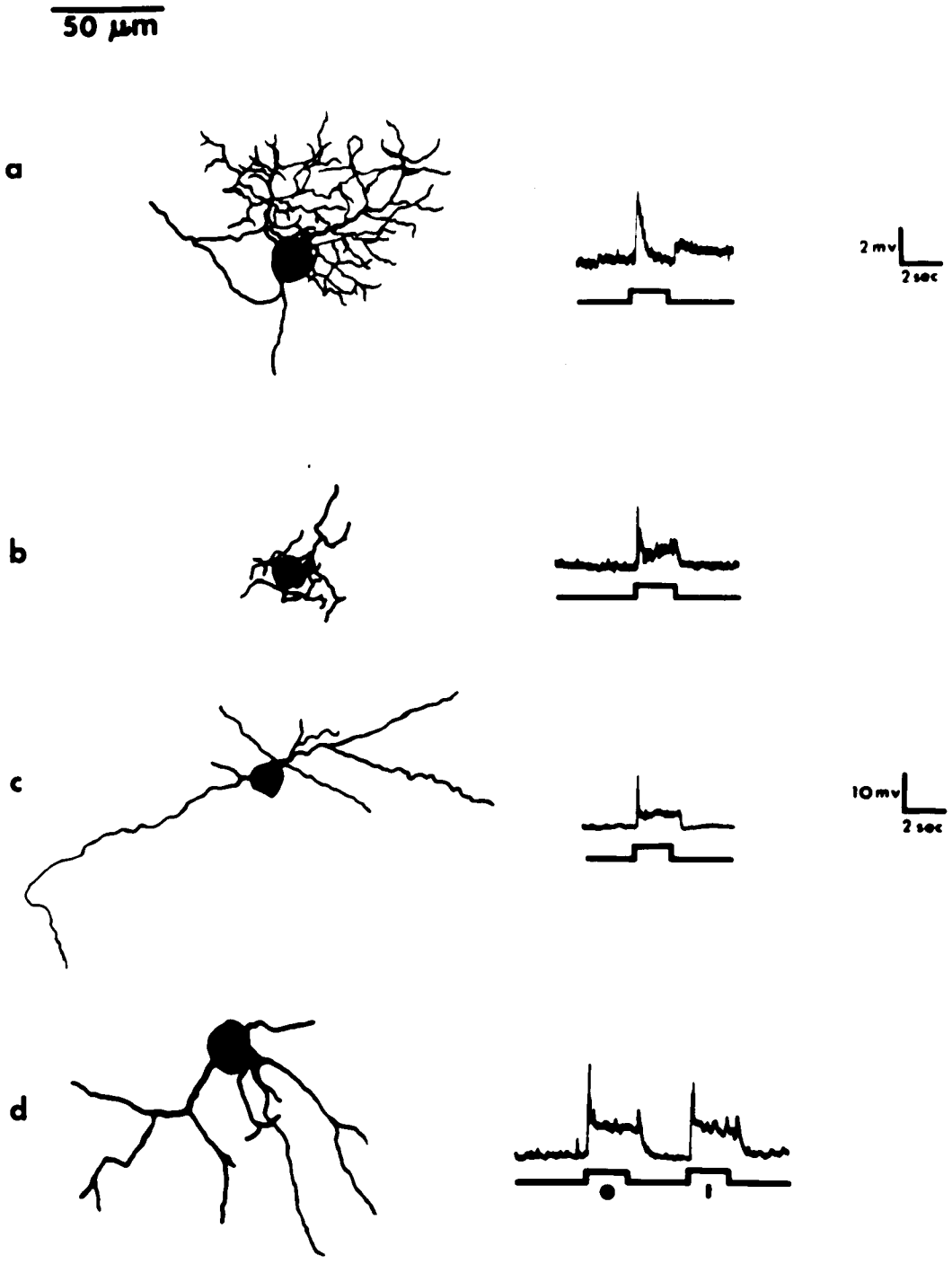

50 µm
a
2 mv
2 sec
b
c
10 mv
2 sec
d
I

Figure 22. Photomicrograph of the flat mounted, ON amacrine cell illustrated in Figure 21d. In order to have dendrites and cell body (although out of focus) in the same picture, many photographs were taken at different planes of focus and put together as a montage. This cell was difficult to photograph for various technical reasons, but was included because radially sectioned material is also presented. X 1550.

Figure 23. Camera lucida drawings and intracellular responses of 3 displaced ON amacrine cells. All units had monophasic receptive fields. The photic response of the top and bottom cells (a & c) is to diffuse illumination and an 80 *um* slit.

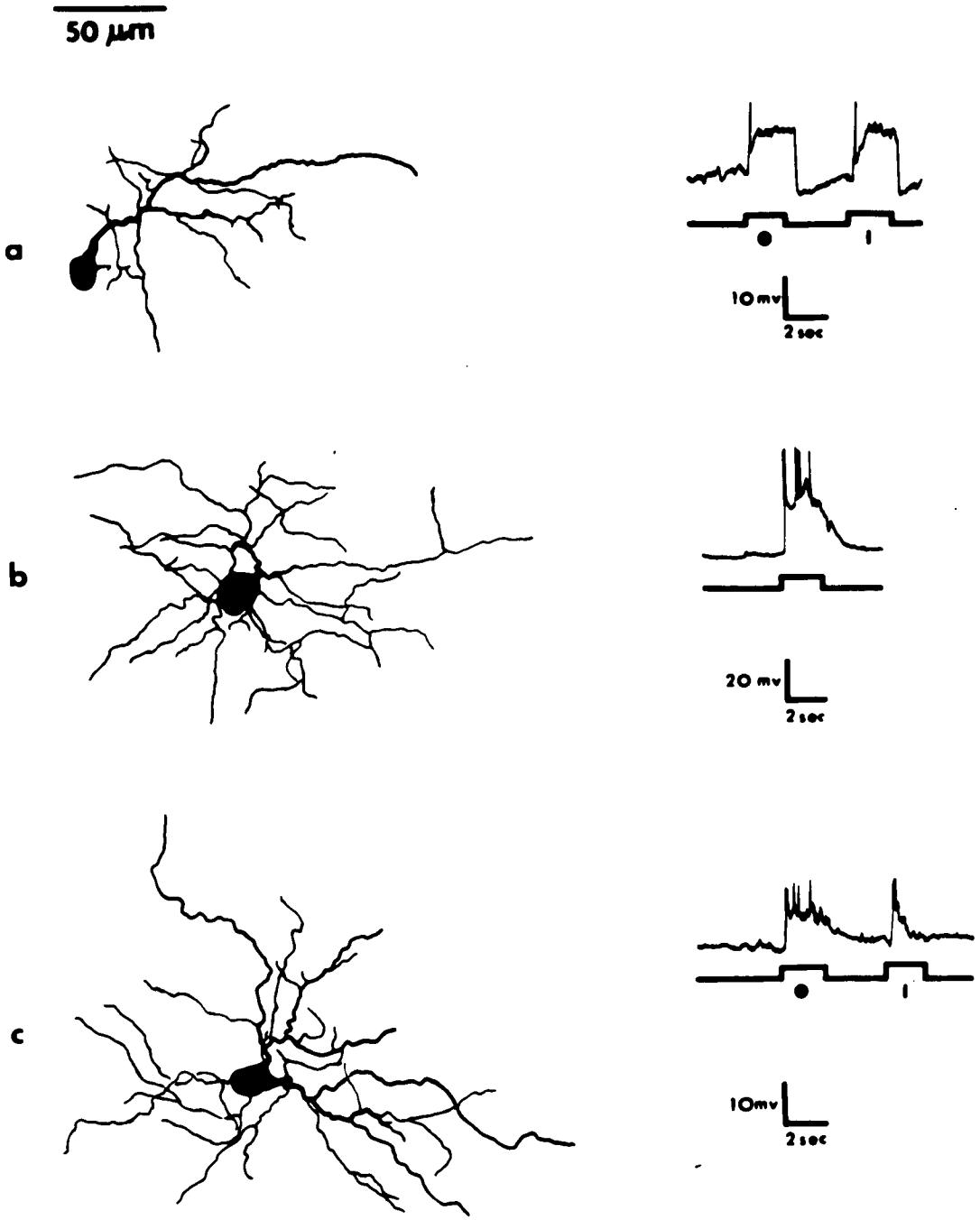

50 µm
a
10 mv
2 sec
b
20 mv
2 sec
c
10mv
2 sec

Figure 24. Photomicrograph of the flat mounted, displaced ON amacrine cell illustrated in Figure 23c. Some of its dendrites and cell body can be seen in one plane of focus. Even though this cell stained rather well, no axon can be demonstrated. X 1960.

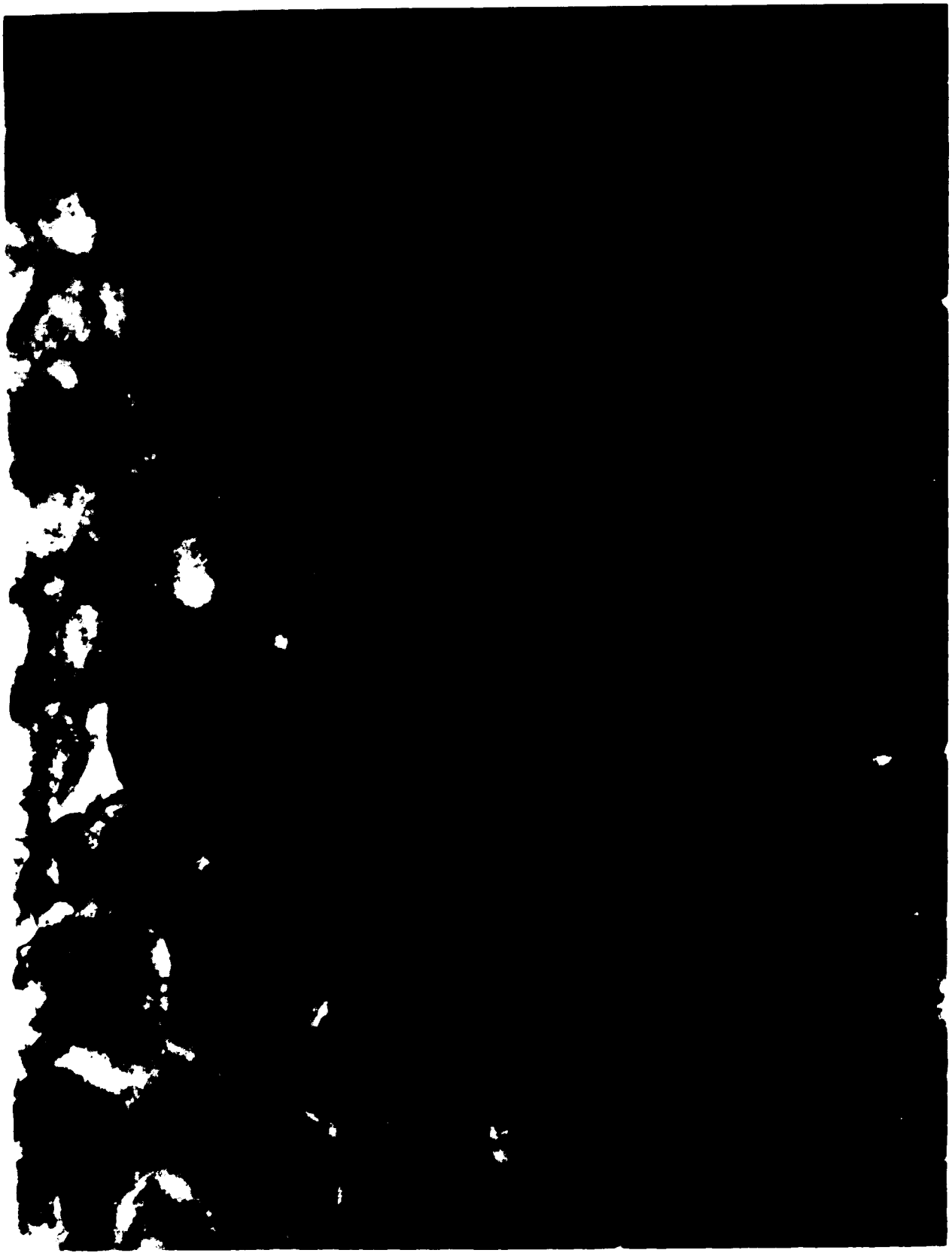


Figure 25. Receptive field plots of an unstained ON (solid line) cell and the cell represented in Figure 23a (dotted line). See text and figure legends 9 & 20 for further explanation.

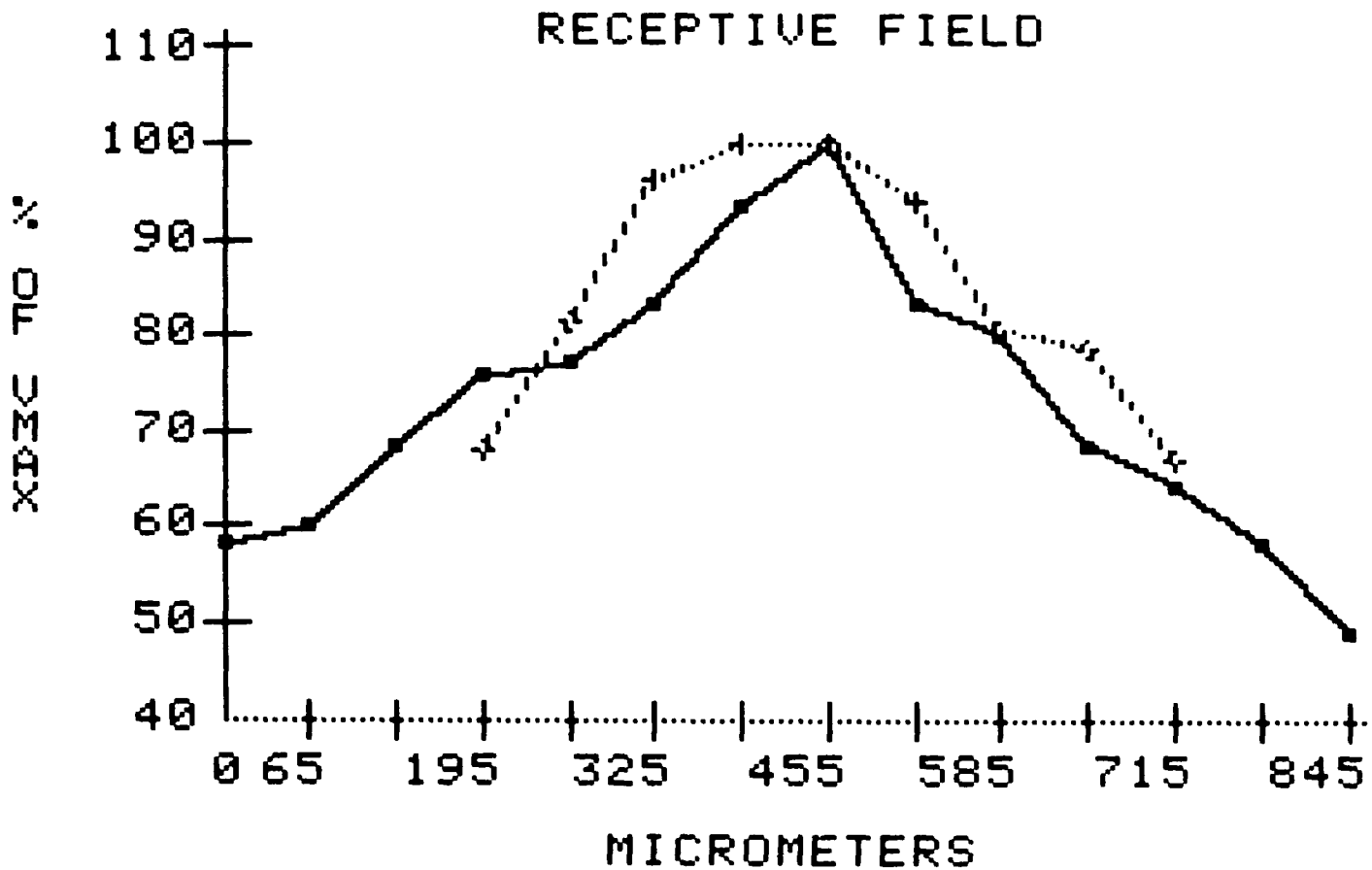

RECEPTIVE FIELD
110
100
90
80
70
60
50
40
0 65
195
325
455
585
715
845
MICROMETERS

Figure 26. Camera lucida drawings and intracellular responses of 2 OFF amacrine cells. The dendrites of the first cell (a) had a few varicosities which appeared as a swelling along the length of the cell's processes and must be seen with high power. The second cell (b) had numerous beaded varicosities which were readily apparent.

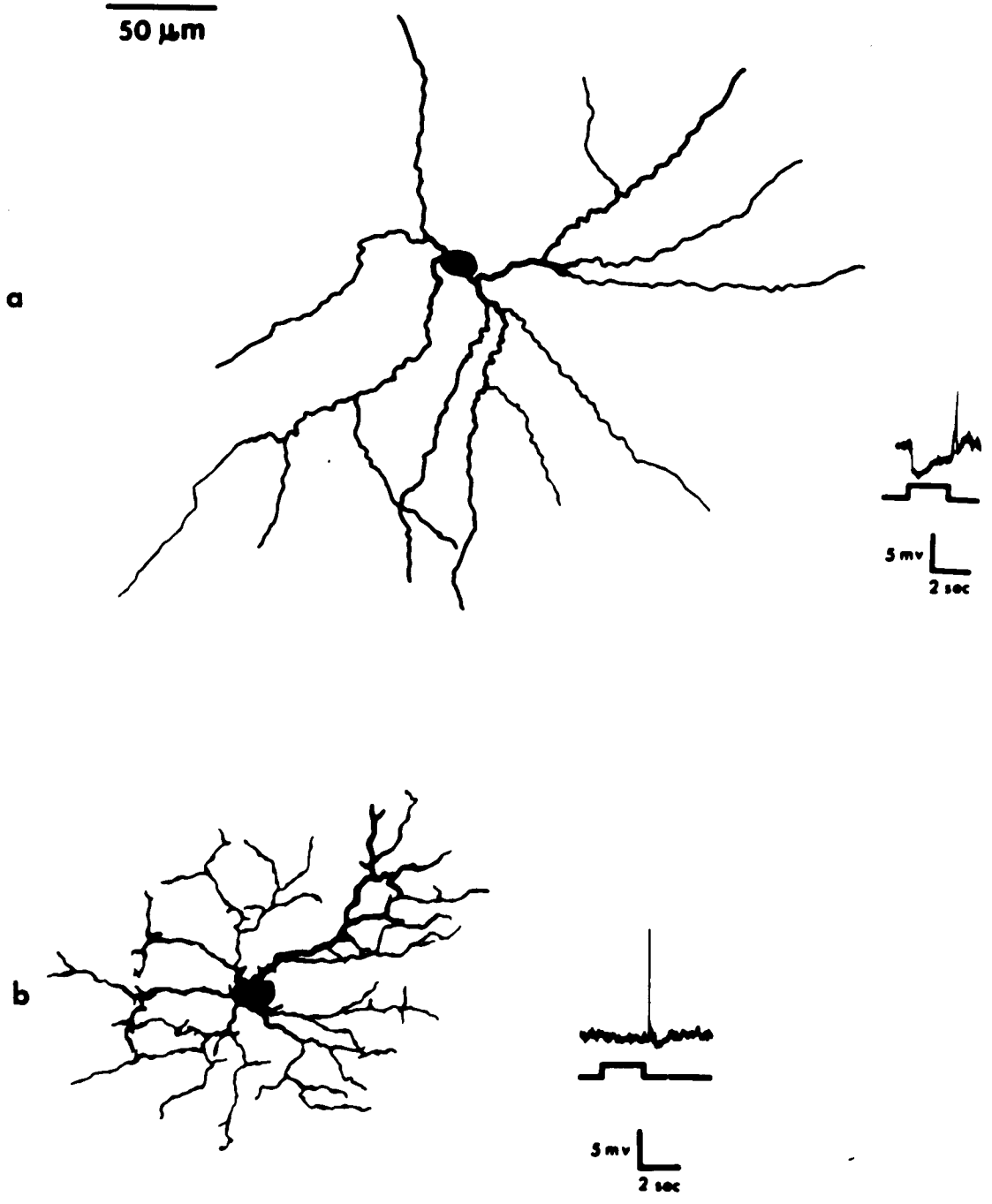

50 µm
a
5 mv
2 sec
b
5 mv
2 sec

Figure 27. Photomicrograph of the flat mounted, OFF amacrine cell illustrated in Figure 26b. Its dendrites and cell body can be seen in one plane of focus. X 550.

Discussion

The present results permit several general conclusions. First in agreement with studies in other species (Kaneko, 1970; Naka, 1977; Bloomfield, 1981; Weiler and Marchiafava, 1981; Nelson, 1982), the mudpuppy retina contains three functionally distinct types (ON, OFF, and ON-OFF) of ganglion and amacrine cells; in addition, ON amacrine cells with their soma displaced to the ganglion cell layer. If nothing else, the heterogeneity of HRP stained cells indicates that there was no obvious bias in cell selection procedures. Many of the previous morphological findings obtained by intracellular staining in mudpuppy (Werblin and Dowling, 1969; Zimmerman and Dowling, 1979) were replicated in this study, and in addition, displaced ON amacrine or the OFF amacrine cells were identified.

Second, in addition to excitatory responses components, some of each class of inner retinal neurons showed prominent IPSPs, including ON and ON-OFF amacrine cells. Zimmerman and Dowling (1979) also observed ON-OFF IPSPs in an amacrine cell. The presence of ON-OFF IPSPs would be expected considering the complex serial and reciprocal synaptology, so distinctive within the inner plexiform layer (Dowling and Werblin, 1969; Dowling, 1979) and although not proven, it is likely that some of these connections would involve ON-OFF amacrine cells. Therefore, if one assumes with Werblin

(1979) and Miller (1979; also see appendix) that ON-OFF amacrine cells are inhibitory, then such IPSPs are possible. One reason for many investigators failing to observe ON-OFF inhibition in these neurons is that chloride in many amacrine cells is passively distributed (Miller and Dacheux, 1976e) and the *ECI* is approximately equal to the dark resting potential. If this is the explanation, one would anticipate that measurements of the input resistance, with high temporal resolution, would show transient decrements of membrane resistance not attributable to EPSPs and spikes alone.

Third, the present description shows a diversity in morphology that generally bears little relationship to physiology. This is not a unique finding. For example, by means of flat mounted Golgi stained neurons, 54 separate categories of inner retinal neurons have been noted (Kolb, Nelson, and Mariani, 1981). Depending upon the physiological criteria adopted, physiologists can describe little more than 1/3 as many exclusive categories. This difference becomes even more perplexing when direct comparisons are made between a cell's electrical response and its morphology made possible with intracellular injections of stain. For example, the ganglion cells of the catfish retina can be electrophysiologically classified according to a number of parameters, including sophisticated receptive field characterization by white noise analysis. Even after years of successful ganglion cell recovery, Naka maintains that he can-

not safely ascribe morphological details to any particular function (Davies and Naka, 1980).

As pointed out by Kolb et al. (1981) the relationship between receptive field and dendritic field size is usually ASSUMED to be highly correlated and actually may not be justified. Although there is some correspondence between these two features, the fit is not as close as one might expect. A number of investigators have reported that in a some cases, a neuron's receptive field is significantly larger than the dendritic fields observed (Boycott and Wassle, 1974; Chan and Naka, 1980). The most satisfactory explanation is to consider that there is input from adjacent units. But this hypothesis must be somewhat stretched to account for those cells which have been demonstrated to have receptive fields smaller than their dendritic fields (Nelson, Famiglietti, and Kolb, 1978; Chan and Naka, 1980)

The lack of correlation between structure and function is not only present in the retina. Jahnsen and Llinas (1982) find that in over 300 intracellularly stained units recorded from various nuclei of the thalamus display essentially the same electrical properties in spite of vast structural differences. They maintain that the cells of the thalamus are "electrophysiologically uniform". Friedlander, Lin, Stanford, and Sherman (1981) have intracellularly stained and recorded from neurons within the lateral geniculate nucleus. They only describe relationships between cell size and the cluster of physiological characteristics of X-,

Y-, and W- cells. Even so, the stained examples of X- and Y- cells overlap adjacent classes of the three categories of neurons determined by golgi staining techniques (Guillery, 1966) within the A and A' laminar of the cat LGB. Other parameters such as ON or OFF seem to have no anatomical correspondence in the cat LGB.

Nevertheless, significant advancements have been made. Functional characteristics such as ON vrs. OFF have been demonstrated to depend upon the level of dendritic stratification within the inner plexiform layer (Famiglietti, Kaneko, and Tachibana, 1977). In addition, a cell's dendritic architecture has also been shown to influence the electrotonic spread of current (Rall, 1969; Miller and Rall, 1982). Other anatomical / physiological correlations can be found between cell body size and the cluster of characteristics that are associated with the retinal X- or Y- system (Lennie, 1980).

Several hypotheses could be examined linking structure to function. For example, one might propose that ganglion cells are constructed to maximize the utilization of neural information passed radially through the inner plexiform layer; amacrine cells might be designed to intercept local information and spread it laterally. Physiologically, ganglion cells display relatively strong excitation when the photic stimulus is small and punctate rather than diffuse. This has been demonstrated using area / intensity threshold experiments (Wiesel, 1960) or through direct observations of

post synaptic potentials (Norman and Werblin, 1974; Wunk and Werblin, 1979). This is in contrast to most amacrine cells, where there is little difference between large and small spots. In fact a stimulus that maximally depolarizes mud-puppy ON-OFF amacrine cells (and hyperpolarizes ganglion cells) is a spinning "optical windmill" which sweeps around the periphery of the cell's receptive field (Thibos and Werblin, 1978). These observations are in agreement with the limited receptive field mapping of the present study using a narrow slit.

It would be interesting to contrast amacrine and ganglion cells and determine if the organization of the dendrites of the former, considering the complex of fiber sizes, special appendages, and synaptology, would favor the laterally oriented distribution of current. For example, gap junctions have been identified between amacrine cells (Dowling, 1979; Kolb, and Nelson, 1981) and have been demonstrated to be functional (Naka and Christensen, 1981). Therefore, situations favoring the lateral spread of current would be of advantage in this type of neuron which has both propagated and graded potentials. Additional differences might also be found between amacrine and ganglion cells by study of the manner in which current is distributed within these neurons. This is done by finding the rate in which they accept an electrical charge and then comparing it to the dendritic geometry. This is a discipline that is only recently advancing (hippocampal pyramidal cells, Turner and

Schwartzkroin, 1980; olfactory bulb mitral cells, Mori, Nowycky, and Shephard, 1981; retinal amacrine cells, Miller 1979b; and spinal lower motor neuron, Fleshman, Burke, Glenn, Len-Tov, and Miller, 1982).

For example Miller and Rall (1982) maintain that certain dendritic features assist in synaptic efficacy. In essence any morphological feature which will increase the effective capacitance of the dendrite will also increase the contribution from spatially restricted changes in the synaptic conductance; specifically, the extension of dendritic length beyond the last synaptic contact. This extra length of dendrite would act as a capacitive "bucket" and increase the efficiency of the passive distribution of charge. Although not the subject of their paper, they also inferred that dendritic appendages such as spines and stalks or varicosities might also accept and store synaptic charge, while also restricting the associated decrement in membrane resistance to this area. In their flat mount paper, Kolb, et al., (1981) have described certain wide field amacrine cells (their A17, A18, A19, amd A22) with long and solitary dendrites (<1mm) that also have occasional varicosities. In some of these cells, synaptic contact is only made at these specialized appendages and might be good candidates to test for the efficient transfer of electrotonic charge across the retina.

Chapter IV

FUNCTIONAL LAMINATION OF THE INNER PLEXIFORM LAYER OF THE MUDPUPPY RETINA

Introduction

The classic Golgi studies of Cajal (1893) documented the existence of many retinal cell types and suggested the five layer stratification of the inner plexiform layer. The functions associated with these five laminae remains largely undiscovered, however, anatomical correlates of the physiologically defined ON and OFF systems have been found. Chronologically, the picture which is emerging started with the early studies of unit recording in the retina. Hartline (1938) first established that the ganglion cell responses in the vertebrate retina can be functionally divided into ON and OFF categories. Subsequently, Werblin and Dowling (1969) functionally identified two types of bipolar cells and since then, several different physiological techniques have been used to establish that the depolarizing and hyperpolarizing bipolars, respectively, provide the excitatory input for ON and OFF responses in ganglion cells (Naka, 1977; Miller, 1979).

The interrelation between morphology and the functionally distinct ON and OFF pathways was first made by

Famiglietti and Kolb (1976), who synthesized their observations of Golgi stained cat retinas with previous investigations of synaptology and physiology. Boycott and Kolb (1973) described two types of cone - bipolars in the mammalian retina which receive their photoreceptor input exclusively from either invaginating or flat synapses. The ultrastructure of these synapses were sufficiently distinct to hypothesize operational differences between them (Raviola and Gilula, 1975). For example, synaptic vesicles cluster around, and can be found fused to the presynaptic membrane of the invaginating synapse indicating transmitter release by exocytosis. In contrast, synaptic vesicles do not cluster at either side of the flat or basal synapse, where transmitter release may occur in a continuous fashion by means of active transport. Famiglietti and Kolb found that flat bipolar cells terminated on the scleral side of the inner plexiform layer (a-sublamina) while the invaginating bipolars terminated on the vitreal side (b-sublamina). The assignment of function was initially made by correlating the receptive fields of extracellulary recorded ganglion cells (those of Cleland and Levick, 1974) with the dendritic fields of their Golgi stained ganglion cells. The extracellular spike data showed that transient OFF center ganglion cells had larger receptive fields than transient ON center cells, while morphological study showed that unistratified ganglion cells, with large perikarya, had larger dendritic fields in the a-sublamina than cells that project-

ed their dendrites only to the b-sublamina. They deduced that cells which synapse in the a-sublamina are concerned with OFF responses, while cells which synapse in the b-sublamina are concerned with ON responses. This led to the conclusion that the flat synapses of cone bipolars are sign conserving, while invaginating synapses are sign inverting. Intracellular staining soon verified this hypothesis in carp (Famiglietti, Kaneko, and Tachibana, 1977) and cat (Nelson, Famiglietti, and Kolb, 1978).

A growing number of studies in a variety of species have looked for functional stratification in the inner plexiform layer by means of intracellular recording and staining (Weiler and Marchiafava, 1981; Bloomfield, 1981). Although the amount of supportive data for this sublamination pattern has grown, so have the number of exceptions. In the cat, a hyperpolarizing bipolar cell, which has been associated with exclusive rod input, "incorrectly" terminates in the b-sublamina (Nelson, 1982); additionally, some sustained amacrine cells have also been found to terminate in the wrong sublamina (Kolb and Nelson, 1981). In turtle, Weiler and Marchiafava (1981) classify ganglion cells into two types. Type A ganglion cells are those which stratify in the expected manner, while type B cells are more diffuse, not adhering to any obvious pattern of stratification. They also found that even though their ON bipolars terminate in the b-sublamina, some OFF or hyperpolarizing bipolars have been found to send their axonal process to the incorrect

b-sublamina as well. Also in turtle, a class of small bipolars have been found to contact both rods and cones by means of both flat and invaginating synapses (Dacheux, 1982), thereby casting some doubt on the functional difference of these contacts. Finally, in catfish, no sublamination pattern has been observed within the inner plexiform layer (Davies and Naka, 1980), although this finding may relate to the low position of this species on the phylogenetic scale.

The present study provided a unique opportunity to interrelate morphological and physiological characteristics within the vertebrate retina. There is only one published abstract that indicates that mudpuppy retinal neurons generally adhere to the usual pattern of sublamination (Zimmerman and Dowling, 1979; also personal communication). As exemplified by the results of the previous chapter, Horseradish peroxidase filled electrodes provide superior anatomical as well as physiological data. The results presented in this chapter confirm and extend the observations of Zimmerman and Dowling, that the excitatory responses in amacrine and ganglion cells adhere to the lamination pattern first suggested by Famiglietti and Kolb (1976) in cat. Chapter V considers the interrelationship between inhibition, excitation, and morphology.

Methods

The techniques of mudpuppy retina perfusion, photic stimulation, intracellular recording, and histology have been previously described in Chapters II and III. The following is concerned with the assignment of dendrites to their relative position within the inner plexiform layer of the mudpuppy retina. Most of these results stem from observations in the flat mount retina employing techniques similar to other investigators (Kolb, Nelson, and Mariani, 1981; Stone, 1981, pp. 86-87). In comparison with the usual sectioned material, this preparation provides a view of all dendrites emanating from a given cell body, allows observations of dendrites which would be perpendicular to the microtome knife and avoids possible tissue damage resulting from futher histological processing. The flat mount method, however, does not as clearly define the boundaries of the inner plexiform layer as radially sectioned material; for this reason, several retinae (e.g., see figure 3) were so processed.

The present strategy involved measuring the depth of cellular details that were clearly stained. This was accomplished by noting the differences between settings on the focus knob on the microscope between objects of interest when clearly in view. Initially, the depth of each major dendrite, along various points, was determined by measuring

the distance from the unstained ganglion cell layer. This technique was abandoned for two reasons: first, such detailed descriptions of the cell's dendrites were not needed. Second, on some retinas the unstained ganglion cell layer could not always be reliably measured due to a variety of problems. The adjustment of the apertures on the microscope which were optimal for finding fine dendrites were usually not effective for visualizing unstained cells. In addition, the ganglion cell layers of different retinas were not uniform in their visibility. On some retinas, even the unstained bundles of ganglion cell axons could be clearly seen with minimal effort, although in others, the unstained ganglion cells and fiber bundles could only be distinctly seen using phase contrast optics.

The distance from the soma to the closest dendritic branching and the most extreme processes was measured for each cell. The values obtained for each cell were then organized, according to cell body location and the projection into the inner plexiform layer was estimated. For amacrine cells (with conventionally located perikarya), the average of the extremes of the dendritic field was used. While for ganglion cells and displaced amacrine cells the midpoint of the dendritic field was also used, but was subtracted from 20 um, the assumed value of the inner plexiform layer. In most cases, these measurements were made with a standard light microscope (Nikon) at 400X, using a 480 nm interference filter, and with the apertures wide open for the short-

est depth of field possible. Differences of approximately 4 um could be reliably detected; however if there was doubt about a measurement, the same procedure could be adopted using 1000x power with an oil emersion lens.

Data presented in figure 32 were obtained from a depolarizing bipolar cell's flat mount measurements that were obtained only using 1000X magnification, at key points along the cell. To better visualize this information, the X, Y, and Z coordinates of the axon terminal were manually keyed into an Apple II microcomputer. By adapting commercially available software (Bill Budge's 3-D Graphics System and Game Tool), the axon terminal was displayed as a computer graphic and then rotated 90 degrees from its original orientation. The cell body was then added to aid in the establishment of the correct orientation. The positioning of inner plexiform layer was established on the basis of the average distance from the unstained amacrine and ganglion cells.

Verification of flat mount measurements was made by comparison with radially sectioned material. Comparison of both types of data were done with an ON amacrine cell (figure 31), which was embedded in Epon and sectioned at 6 um. The similarity between these results suggest that minimal error and distortion were encountered by the use of the flat mount procedure.

Results and Discussion

When the flat mounted retina was viewed under the microscope and gradually brought into focus, its 3- dimensional structure became quite evident, specifically the segregation of dendrites from the ON and OFF systems. Since the dendrites of ON ganglion and displaced amacrines cells were localized nearer the vitreal surface of the inner plexiform layer, their somas and the majority of the dendrites could be viewed with little or no adjustment of the focus. In the case of OFF ganglion cells, however, such simultaneous visualizations are impossible since the dendritic fields are placed closer to the inner nuclear layer. For similar reasons, the dendritic fields and cell bodies of OFF (but not ON) amacrine cells could be simultaneously visualized.

Quantitative Observations

Table 1 summarizes all quantitative information obtained from 26 cells. These data taken from the flat mount measurements demonstrates that all ON as opposed to OFF units send their respective dendrites toward the vitreal or scleral surface of the inner plexiform layer. The dendrites of the ON-OFF units did not favor one side or the other. This does not mean that the inner plexiform layer of the

mudpuppy retina follows the strict pattern of lamination found in the cat retina (Nelson, Famiglietti, and Kolb, 1978). The inner plexiform layer of the mudpuppy retina is not divided by a network of capillaries which serves as the boundary between sublaminations as for those retinas with a supplementary retinal circulation.

The differences between the ON and OFF systems are illustrated by several graphic comparisons. Figure 28 compares the estimated center of the dendritic fields of 12 ON and 4 OFF cells. These data include both conventionally placed and displaced amacrine cells as well as ganglion cells. With the measurements starting at the scleral side of inner plexiform layer, the ON units cluster around a mean of 13.5 *um* (S.E. = 0.64) whereas the OFF units had a mean of 5.8 *um* (S.E. = 1.56). A 1-tailed t-test for independent means estimates that this difference is statistically significant, assuming the usual thickness of the inner plexiform layer is 20 *um* ($t=5.5$, $df=14$, $p<0.0005$). This difference would still remain significant ($p<0.025$) if the thickness of the inner plexiform layer was taken at 15 *um*, a value 25% thinner than the lower estimate reported by Dowling and Werblin (1969).

Figure 29 compares the most disparate extent of dendritic fields for all the cells in table 1. The top graph shows data from cells with their perikarya in the ganglion cell layer (including displaced amacrine cells), while the bottom one shows data from conventionally located amacrine

cells. In both cases the ordinate is the distance from the cell body to the first viewing of dendrites (solid bars) and the final viewing of dendrites (striped bars). ON ganglion and OFF amacrine cells have their dendrites closer to their cell bodies, as opposed to ON amacrine and OFF ganglion cells which have them further away. Thus, the upper graph, reflects distance from the ganglion cell layer while the lower graph, reflects a distance from the inner nuclear layer. ON ganglion cells, displaced ON amacrines, and OFF amacrine cells have their dendrites closer to their cell bodies than do conventionally placed ON amacrines and OFF ganglion cells. This graphic comparison also shows that ON-OFF amacrine and ganglion cells have processes in both sublamina which covers a much larger area within the inner plexiform layer than ON or OFF units.

Photographic Visualization

Figure 30 shows photographs of 3 cells (from one retina) taken at 2 different planes of focus. In both pictures, from left to right, is an ON-OFF ganglion, an ON ganglion, and an OFF ganglion cell. All have identifiable axons and correspond to the camera lucida drawings of figures 6a, 11b, and 13a. The upper photograph shows the dendrites of the ON cell, along with the axon hillock of the ON-OFF ganglion cell to the left, while the dendrites of the

OFF cell, to the right, are blurred. The bottom photograph was obtained in the identical manner save that the microscope focus was advanced 14 *um* toward the sclera. When the focus was so adjusted, the dendrites of the OFF ganglion cell (to the right) are the only processes appearing in clear view. Due to the depth of field properties of the objectives on the microscope, only the units that send their processes a short distance can have their cell bodies and most of their dendrites photographed within the same plane of focus. The serendipitous impalement and successful recovery of these functionally distinct neurons is perhaps the most direct evidence of the segregation of their dendrites.

Radial Sectioned Data

Figure 31 represents two adjacent sections of the ON amacrine cell which was presented in figures 21d and 22. This cell was embedded in Epon Araldite and sectioned at 6 *um* and photographed using phase contrast optics to improve visual discrimination. The right picture shows the cell body with a dendrite coursing vitreally. The left photograph shows the adjacent section (which was flipped over when mounted on the slide) where the continuation of the dendrite segment can be seen running under the ganglion cell layer. Another dendrite was found (not illustrated), starting about a third into the inner plexiform layer which also

projected vitreally. Whether viewed in flat mount or in a radial section, this ON cell which has been closely examined, sends most of its dendrites to the vitreal surface of the inner plexiform layer. The measurements obtained from this cell by means of flat mount or sectioned material agreed within 10%, thus validating the more complete and easier flat mount procedure.

Bipolar Cells

Data from 2 depolarizing bipolar cells were also collected and it was determined that the axon terminals of these cells projected toward the vitreal surface of the inner plexiform layer. To show a visual summarization of the flat mount measurements, one of these cells was drawn in its normal orientation (upper left corner of figure 32) and as an orthogonally rotated computerized reconstruction (middle). The dotted lines represent the usual 20 micron thickness of the inner plexiform layer. This shows that the axonal processes of this cell, which is the dominant source of excitation within the ON system, are also found at the vitreal surface of the inner plexiform layer. Although intracellular recordings were obtained from hyperpolarizing bipolar cells, none were successfully stained.

In summary, the dendrites of mudpuppy ON and OFF inner retinal cells have been demonstrated to adhere to the previ-

ously described pattern of laminar organization in three ways: statistical analysis, photographic utilization of depth of field, and the radial sectioning of a cell. This analysis was based on the physiological characterization of these cells by their excitatory PSPs alone. No blatant exceptions to this pattern were found, but it is assumed that this only reflects random sampling, nevertheless there is overlap between ON and OFF cell dendrites. This indicates that there can be a structural basis for interactions between ON and OFF systems. In that, many of these units display either ON and/or OFF IPSPs which do not necessarily correlate with the laminar character of inner plexiform layer.

Figure 28. Graph demonstrating the spatial density of the dendritic fields of 12 ON and 4 OFF cells (both amacrine and ganglion cells). On the ordinate are the estimated centers of the dendritic fields within the inner plexiform layer, where 0 represents the most scleral boundary and 20 the most vitreal. Like retina of the cat, ON cells primarily occupy the vitreal 3/5 of the inner plexiform layer, with OFF cells more scleraly located.

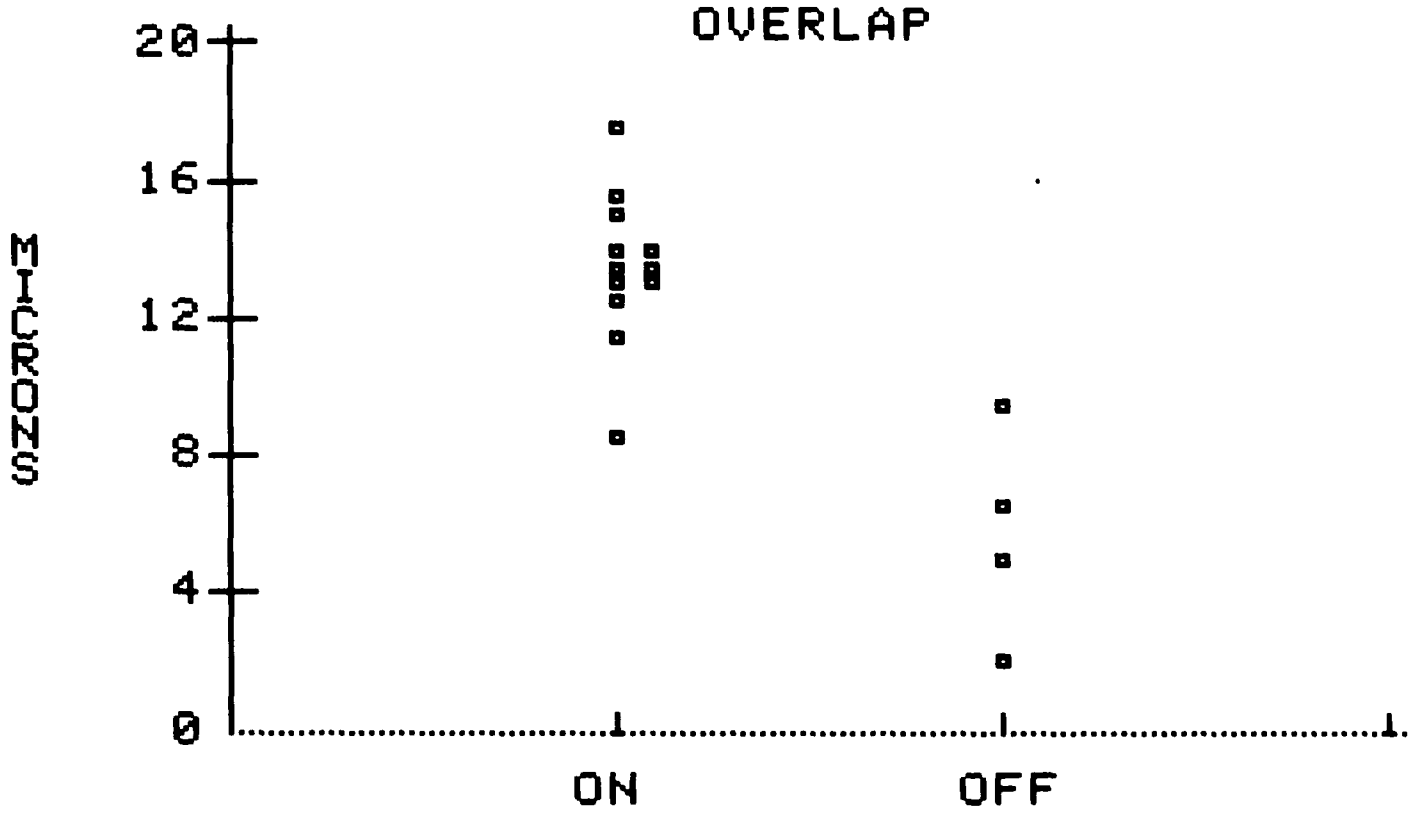

OVERLAP
MICRONS
20
16
12
8
4
0
ON
OFF

Figure 29. Bar graph showing the extremes of the dendritic fields of the cells in table 1. For both sets of coordinates, the ordinate value indicates the distance from the cell body to the closest and furthest dendrites. The upper graph summarized cells with their soma within the ganglion cell layer (displaced ON amacrine and ganglion cells), 0 and 20 *um* on this ordinate respectively indicate the vitreal and scleral boundaries of the inner plexiform layer. The lower graph summarized cells with their soma within the inner nuclear layer (conventionally placed amacrine cells), 0 and 20 *um* indicate the scleral and vitreal boundaries of the inner plexiform layer. See text for further explanation.

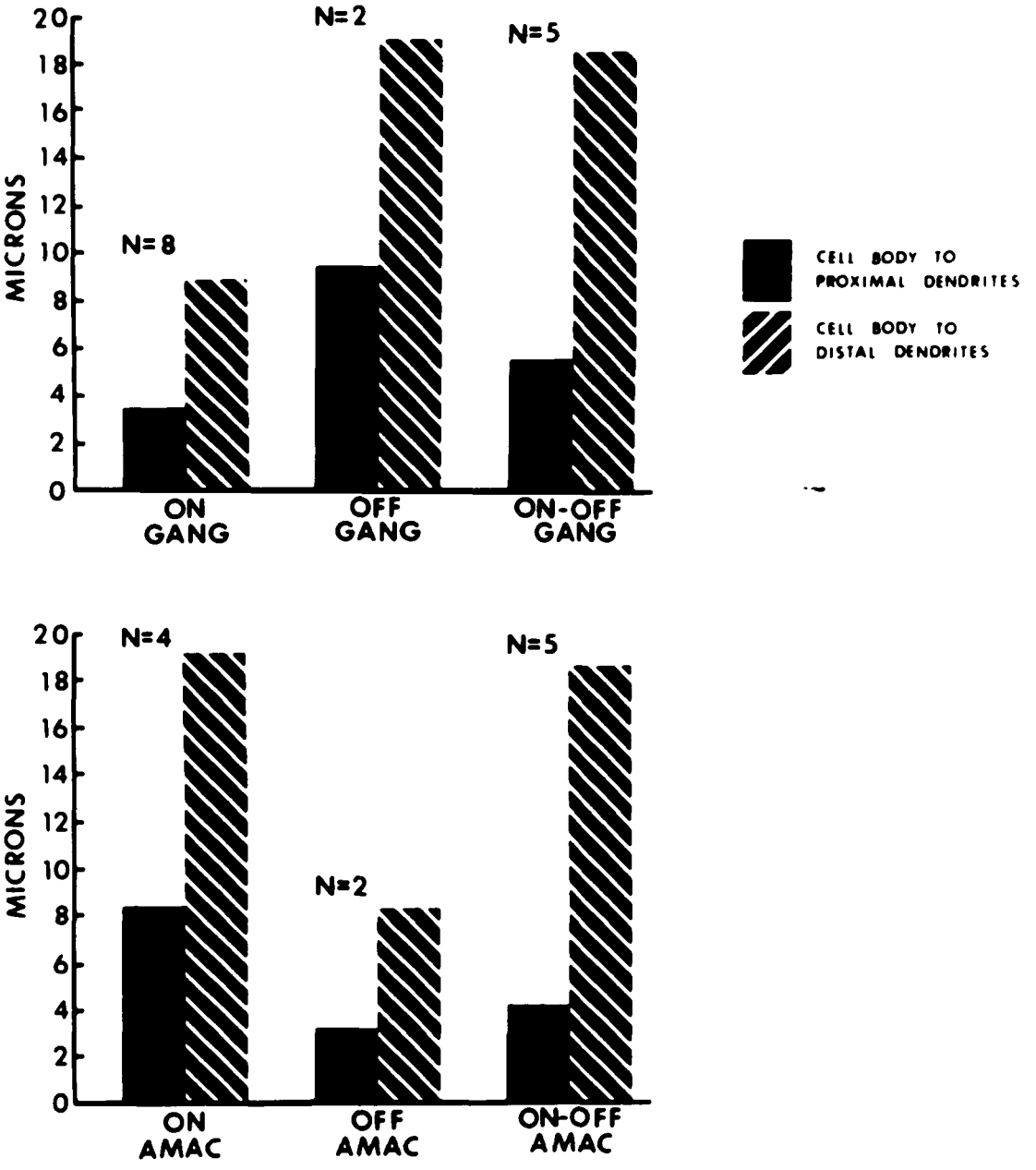
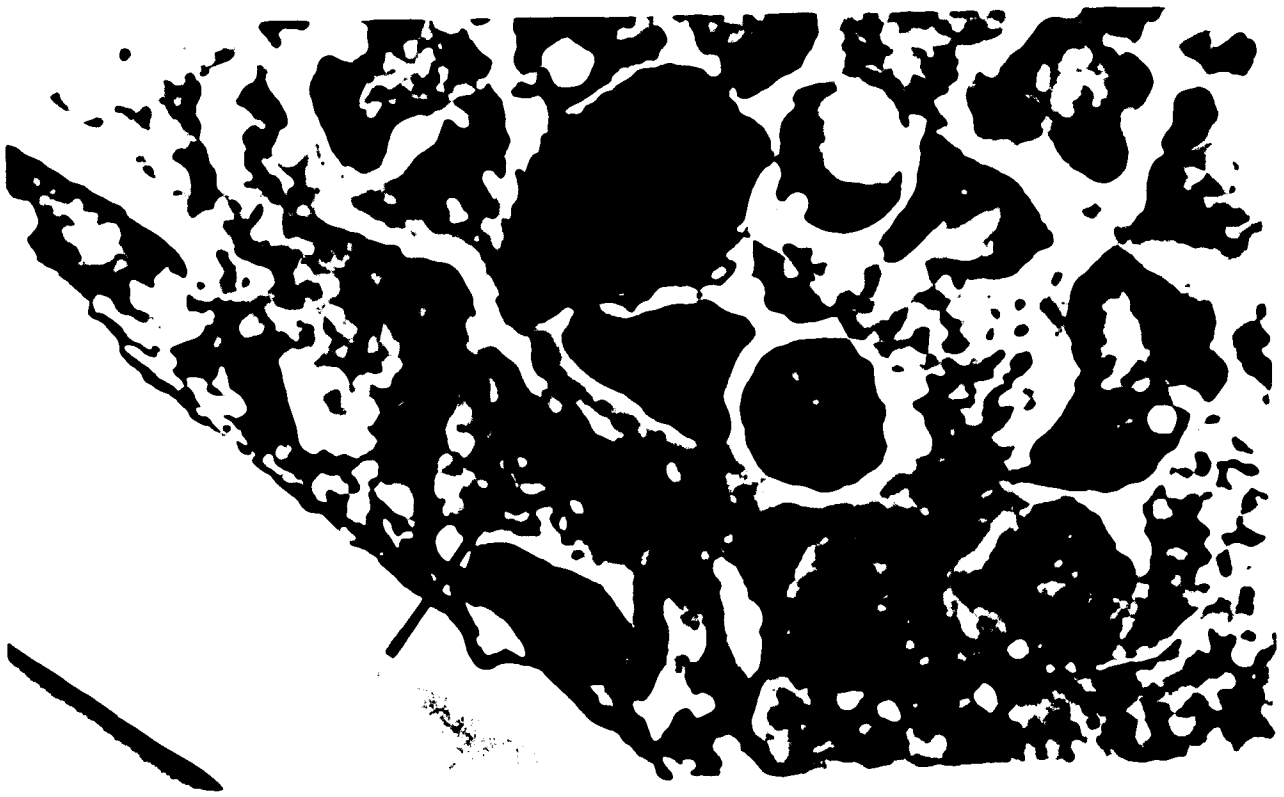

20
18
16
14
12
10
8
6
4
2
0
MICRONS
N=8
N=2
N=5
ON GANG
OFF GANG
ON-OFF GANG
CELL BODY TO PROXIMAL DENDRITES
CELL BODY TO DISTAL DENDRITES
N=4
N=2
N=5
ON AMAC
OFF AMAC
ON-OFF AMAC

Figure 30. Light micrograph of three ganglion cells shown at two different planes of focus 14 *um* apart. In the upper photograph, the axon of the ON-OFF cell (left) can be seen along with the dendrites of the ON cell (center), while the dendrites of the OFF cell (right) remain blurred. In the lower photograph the axon of the ON-OFF cell and the dendrites of the ON cell can no longer be seen, but, the dendrites of the OFF cell are visible. X 380.

Figure 31. Light micrograph of an HRP stained ON amacrine cell from a radially sectioned retina. This same cell is shown, as a flat mount, in figures 21d and 22. The top picture shows the cell body and a segment of dendrite which courses toward the ganglion cell layer. The bottom picture is the adjacent section showing only a small segment of dendrite (see arrow) running parallel to the ganglion cell layer. X 995.



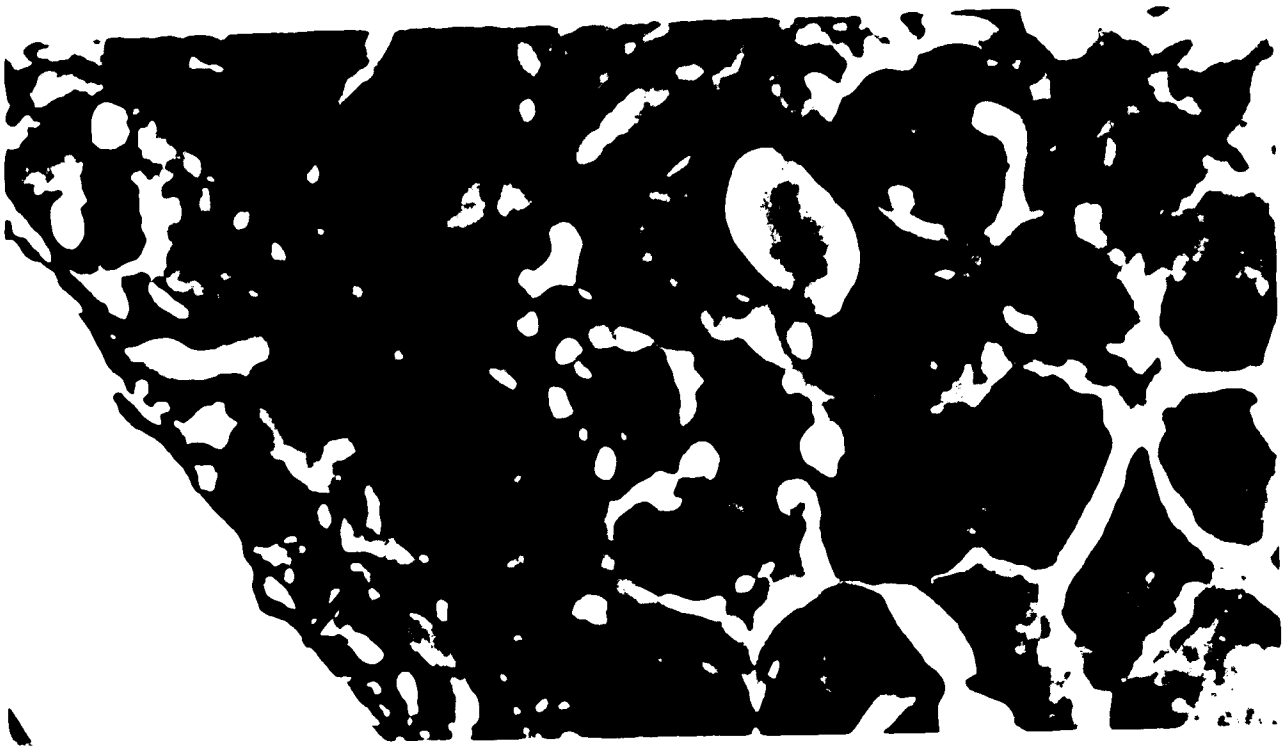
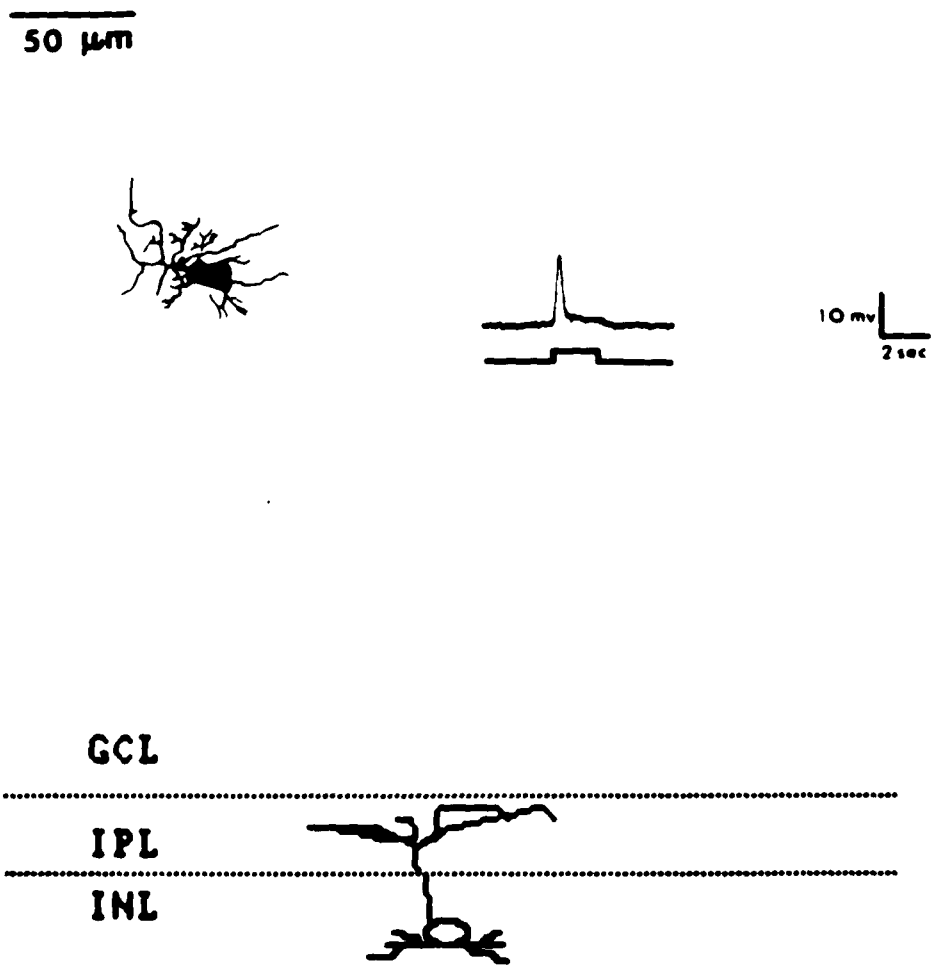


Figure 32. The upper left illustration is a camera lucida drawing of a depolarizing bipolar cell in its flat mounted orientation, with its intracellularly recorded response to a 2 sec duration light flash in the upper right. The lower figure is a computerized reconstruction of this same cell after it was rotated 90 degrees. The horizontal dotted lines indicated the assumed 20 μm thickness of the inner plexiform layer (IPL). GCL and INL represent respectively the ganglion cell layer and the inner nuclear layer.


50 µm
10 mv
2 sec
GCL
IPL
INL

Chapter V

INTERACTIONS OF ON AND OFF POTENTIALS IN ON AMACRINE AND GANGLION CELLS

Introduction

A large body of anatomical and physiological data show that the depolarizing and hyperpolarizing bipolar cells are the primary source of excitation in ON and OFF inner retinal neurons. The results presented in the previous two chapters indicate that in mudpuppy, this excitatory input adheres to a definite pattern of lamination. However, the majority of inner retinal neurons, whether they are classified as ON, OFF, or ON-OFF on the basis of their excitatory responses, also receive inhibitory input. As stressed by many current models of retinal function (see appendix), normal functioning within the inner plexiform layer involves a complex interplay of inhibitory and excitatory mechanisms. Accordingly, the present chapter considers the interrelationship between laminar morphology, and excitation and inhibition in determining the organization of the inner plexiform layer.

A limited number of studies have suggested that there must be some independence between ON and OFF inhibitory potentials. For example, Frumkes, et al. (1981) has shown that certain ganglion cells with ON-OFF IPSPs can have their

OFF IPSP selectively blocked by 0.1 mM of strychnine, while leaving the ON IPSP relatively unaffected. A similar relationship between GABA with ON inhibition and glycine (and/or taurine) with OFF inhibition in rabbit (Cunningham and Miller, 1976) and cat (Ikeda and Sheardown, 1982). Current injection studies of some ON-OFF ganglion cells, with the typical EPSP-IPSP sequence, have found that the IPSPs can have unequal reversal potentials, usually with the ON IPSP reversing at lower current values (Frumkes, Coleman, and Nicotera, 1980). This result is indicative of spatially separate synaptic contacts from ON and OFF amacrine cells, the hypothesized source of the IPSPs of these ganglion cells. The above not only indicates that there are separate ON and OFF inhibitory potentials, but that they might also adhere to the principles associated with the functional segregation of ON and OFF systems. In teleost fish, the dendrites of ON-OFF amacrine cells project to both sublaminae, yet histochemical studies find that inhibitory amino acids are not diffusely found in the inner plexiform layer. Glycine has been found primarily in the scleral portion of the inner plexiform layer (Marc and Lam, 1981), whereas GABA has been located at the vitreal portion (Yazulla, 1981; Marc, Stell, Bok, and Lam, 1978).

Nevertheless, the physiological separation of ON and OFF inhibitory systems seems to be the exception and not the rule. Some pharmacological studies in cat (Kirby and Enroth-Cugell, 1976; Kirby and Schweitzer-Tong; 1980) and

rabbit (Wyatt and Daw, 1976; Caldwell and Daw, 1978; Caldwell, Daw, and Wyatt, 1978) ganglion cells report no segregation of GABA and glycine into discrete ON and OFF systems, but rather suggest relationships to X- vs. Y- cell types and/or to motion sensivity. In mudpuppy, either GABA or glycine antagonists entirely block ON-OFF IPSPs in the majority of ganglion cell's (Miller, et. al., 1981). Based on current injection and post synaptic potential analysis Frumkes, et al. (1980; 1981) also report that a majority of ganglion cells have the same reversal potential for the ON-OFF IPSPs. A logical candidate to mediate inhibitory interactions throughout the inner plexiform layer would be a multistratified ON-OFF amacrine cell. This cell can receive input from both bipolars and could output to either narrowly or to other multi - stratified neurons. Taken to the extreme, some retinal models state that the exclusive source of inhibition in the inner retina is the ON-OFF amacrine cell (Wunk and Werblin, 1979).

Other results not only question the expected pattern of sublamination but also suggest a much more involved interplay between excitatory and inhibitory input. Frumkes, et al. (1981) report that in the case of some ON ganglion cells, which have both ON and OFF IPSPs, strychnine had blocked only the OFF IPSP. Bicuculline had to be added to the perfusate in order to block the remaining ON IPSP. Other studies have found that in addition to transient IPSPs there is evidence of sustained inhibition in the retina.

Dvorak, Belgum, and McReynolds (1980) maintain that in OFF ganglion cells there is a picrotoxin sensitive, sustained ON IPSP. The same group of investigators have also produced data showing that in many ON ganglion cells, the application of the synaptic blocking agent Co++, can cause a depolarization up to 10 mV. This indicates that there is the dark release of an inhibitory transmitter (Belgum, Dvorak, and McReynolds, 1982). Dark release of transmitter in the inner retina is usually associated with the OFF system.

The foregoing review suggests that the functioning of inner retinal neurons cannot always be described in terms of a simple bilaminar organization, and stresses a complex interplay between inhibition and excitation. The present experiments give a description of this interplay by obtaining physiological, pharmacological, and morphological data from the same preparation. Although it was rarely possible to obtain all three types of data from the same cell, at least two were generally possible. These results will suggest a lack of organization for inhibition within the inner plexiform layer, and stress the interactions between inhibitory and excitatory PSPs in determining the overall responsiveness of these neurons. The post synaptic potentials of units classified as ON cells were studied, and in some cases, these cells were also stained in order to examine their dendritic projection into the inner plexiform layer. Similar results were obtained from OFF and ON-OFF units will be the subject of a future communication.

Methods

The techniques employed have been described in the previous chapters. The concentrations of all substances were those demonstrated to be effective and selective in prior studies using a similar superfused eyecup preparation in mudpuppy (Frumkes, et al., 1981; Slaughter and Miller, 1981). In particular, 10-25 μM picrotoxin effectively blocked GABAergic synapses and had no influence on glycinergic ones; in a similar manner, 10 μM strychnine selectively blocked glycinergic synapses.

Cell selection is made blindly and randomly and therefore, the experimenter had to often make due with less than ideal conditions; especially with the added complication of the electrode being filled with HRP. Ideally, the research strategy was to make receptive field measurements, followed by PSP analysis with current injection and pharmacological experiments, and then inject HRP for the morphology. In actuality this did occur, but not often. Usually the choice of procedure was made in order to take the fullest advantage of the particular unit encountered. Additional data were only collected if the effects of a cell's initial experiments proved reversible and good physiological condition was maintained.

Results and Discussion

Figure 10a shows results from an ON ganglion cell, which characteristically has its dendrites restricted to the vitreal side of the inner plexiform layer. The photic response of this unit is characterized by its EPSP - IPSP sequence at light onset. According to the model of Frumkes, et al. (1981; see appendix) the probable inputs are the depolarizing bipolars and ON amacrine cells. These data show that in at least one case, inhibitory as well as excitatory input can adhere to a bilaminar organization.

Figure 33 shows the results of detailed current injection experiments from a displaced ON amacrine cell, with its dendrites restricted to the vitreal 3/5 of the inner plexiform layer (see table 1 and figure 23b). The top control record shows that the response of this cell is characterized by a sustained depolarization which produces transient ON spiking, but there are also signs of transient ON-OFF hyperpolarizing responses. In conjunction with the photic response, +0.1 nA pulses were presented (second trace) through a resistive bridge device (partially out of balance due to a total resistance greater than 500 megohms). During illumination, the voltage response (resistance x 0.1 nA) to these current pulses is attenuated, indicating a decrease in the cell's input resistance. Note that the decrement is maximal at light onset and offset, indicating transient IPSPs.

Utilization of DC positive current (3rd trace), decreased the size of the sustained depolarization to reveal the transient hyperpolarizations. In contrast, negative DC current (bottom trace) exaggerated the sustained depolarization and inverted the OFF component; the transient ON component was most likely overwhelmed by the exaggerated larger, sustained potential. Assuming a conventional distribution of Na+, K+, and Cl- ions, these data demonstrate transient inhibition at light onset and offset which surround the sustained EPSP. Although not illustrated, the ON-OFF IPSPs of this cell were both attenuated by picrotoxin (25 uM), in addition only GABA (2.5 mM) caused the loss the light evoked response. No change could be detected with prolonged exposure (1.5 min) to an equal concentration of glycine. Collectively, the present results indicate that the response of this stratified ON cell involves an interplay of inhibition and excitation, and that the inhibitory input (quite probably a bistratified ON-OFF amacrine cell) does not challenge the generality of the principles of the functional stratification of the inner plexiform layer.

Frumkes, et al. (1981) observed ON neurons with pharmacological properties similar to the cell in figure 33. They also observed other types of ON cells, including a "hybrid" in which GABA and glycine antagonists had to be used together to block all signs of inhibition. Similar results were obtained from a displaced ON amacrine cell as shown in figure 34. Morphological and receptive field data have al-

ready been presented from this displaced ON amacrine cell (figure 23a and 25 -dotted line) and it exhibits the typical pattern of transient IPSPs surrounding the sustained EPSP. In the control medium, the transient ON hyperpolarization can be seen right after the spike, at light onset. At offset, the IPSP can at times reach 10 mV below the dark potential. The full extent of the OFF IPSP is better observed when the membrane is depolarized by the adaptation of the center of the receptive field with the slit (not illustrated). Under this condition, the diffuse flash slightly increased the ON EPSP and enhanced the OFF IPSP. After obtaining control records, 20 uM of picrotoxin was introduced into the perfusate for 60 sec, which attenuated the IPSP at light onset. This is best observed at the examples provided at the slower sweep speed, as one can see there is a progressive narrowing of the leading edge of the response. At the illustrated traces obtained at the expanded sweep speed (upper right), this loss of ON inhibition is evidenced by a broadening of the initial ON spike. After obtaining this data control ringers was reintroduced for 3.5 min, but the cell had still not fully recovered, as evidenced by the partial failure of the ON IPSP to return in response to focal illumination. Nevertheless, with no change in the overall resting potential, strychnine (20 uM) application had depolarized the transient OFF IPSP an additional 3-4 mV and allowed another spike at light offset to occur. The PSPs at light offset were also examined using APB to simplify the

analysis with the remove all ON activity due to depolarizing bipolar cells (Slaughter and Miller, 1981). As shown in the bottom trace of figure 34, a spike was also observed following the remaining OFF IPSP. While still influenced by APB, depolarizing current greatly enhanced the OFF IPSP and hyperpolarizing current "flipped" this inhibitory potential. Hyperpolarizing current instead of enhancing the remaining OFF spikes eliminated them, indicating they were not generated from synaptic influences, rather, these excitatory potentials may be due to voltage dependent properties of non-synaptic membrane. The data from the cell represented in figures 23a, 25, and 34 indicates, through the differential effects of picrotoxin and strychnine, that some ON cells receive three separate types of synaptic input: a) ON excitation from depolarizing bipolar cells, b) transient ON inhibition from a GABAergic ON amacrine cell and c) transient OFF inhibition from a glycinergic OFF amacrine cell.

Figure 35 shows the results from an unstained ON cell, with a response and receptive field (figure 25- solid line) similar to the unit described in figure 34; however, it had a very deep resting potential of -70 mV. This cell was sensitive to both 2.5 mM GABA and glycine (upper left two traces). Picrotoxin (25 uM) only increased the size of the light evoked response (third trace from top left), possibly due to a general increase in the membrane's resistance (Miller et. al., 1981). Strychnine depolarized the cell by 10 mV and caused the loss of the ON depolarization. A tran-

sient OFF PSP was detected with both depolarizing and hyperpolarizing current in the presence of APB (50 μM), perhaps contributing to the OFF action potential which was observed during strychnine application. Similar depolarizations and attenuation of the photic response were obtained in 2 other ON cells. Unfortunately input resistance, associated with strychnine application, was not tested. These results are similar to those obtained by Belgum, et al. (1982), where it was found that the resting potential of some ON ganglion cells were depolarized by Co++ application. These data indicate that depolarizing bipolar excitation may be modulated by a disinhibitory mechanism from an OFF amacrine and/or an inhibitory hyperpolarizing bipolar cell, again showing interactions between ON excitatory and OFF inhibitory systems. This dark release of an inhibitory transmitter has also served as the basis of the explanation of why many ON cells lack an antagonistic surround (Wunk and Werblin, 1979).

In summary, the present data indicates that ON neurons which restrict their dendrites to the vitreal 3/5 of the inner plexiform layer and receive its excitatory input from only 1 type of bipolar cell can have IPSPs that originate from OFF cells. This OFF inhibition may act in concert with transient ON-OFF IPSPs, however, it must be regarded as autonomous and separate from ON-OFF amacrine cells. Congruent data (ON inhibition in OFF cells) were obtained in an OFF ganglion cell that was hyperpolarized (by current injection) to a resting potential of -130 mV and then exposed to APB.

The APB had the effect of disallowing a sustained ON IPSP and letting the exaggerated disfacilitation become more prominent. Separate ON and OFF IPSPs appear to modulate excitation in OFF and ON tertiary retinal neurons and therefore must be a significant challenge to the generality of the principles associated with the functional lamination of the inner plexiform layer.

Figure 33. Intracellular responses from a displaced ON amacrine cell (shown in figure 23b), in response to diffuse light or an 80 *um* slit. This cell had a resting potential of -70 mV and a monophasic receptive field. The second trace was obtained while +0.1 nA pulses of current were presented through an out of balance resistive bridge. Note that the input resistance (I x R) was lowest at light onset and offset, suggesting the presence of transient ON-OFF IPSPs. Similar results (not illustrated) were obtained with -0.1 nA pulses. These IPSPs are more clearly observed when the cell was depolarized with +0.14 nA of current (third row) and were reversed in polarity when the cell was hyperpolarized with -0.1 nA of current (bottom row). All responses are approximately in line with the stimuli illustrated at the bottom of the figure.

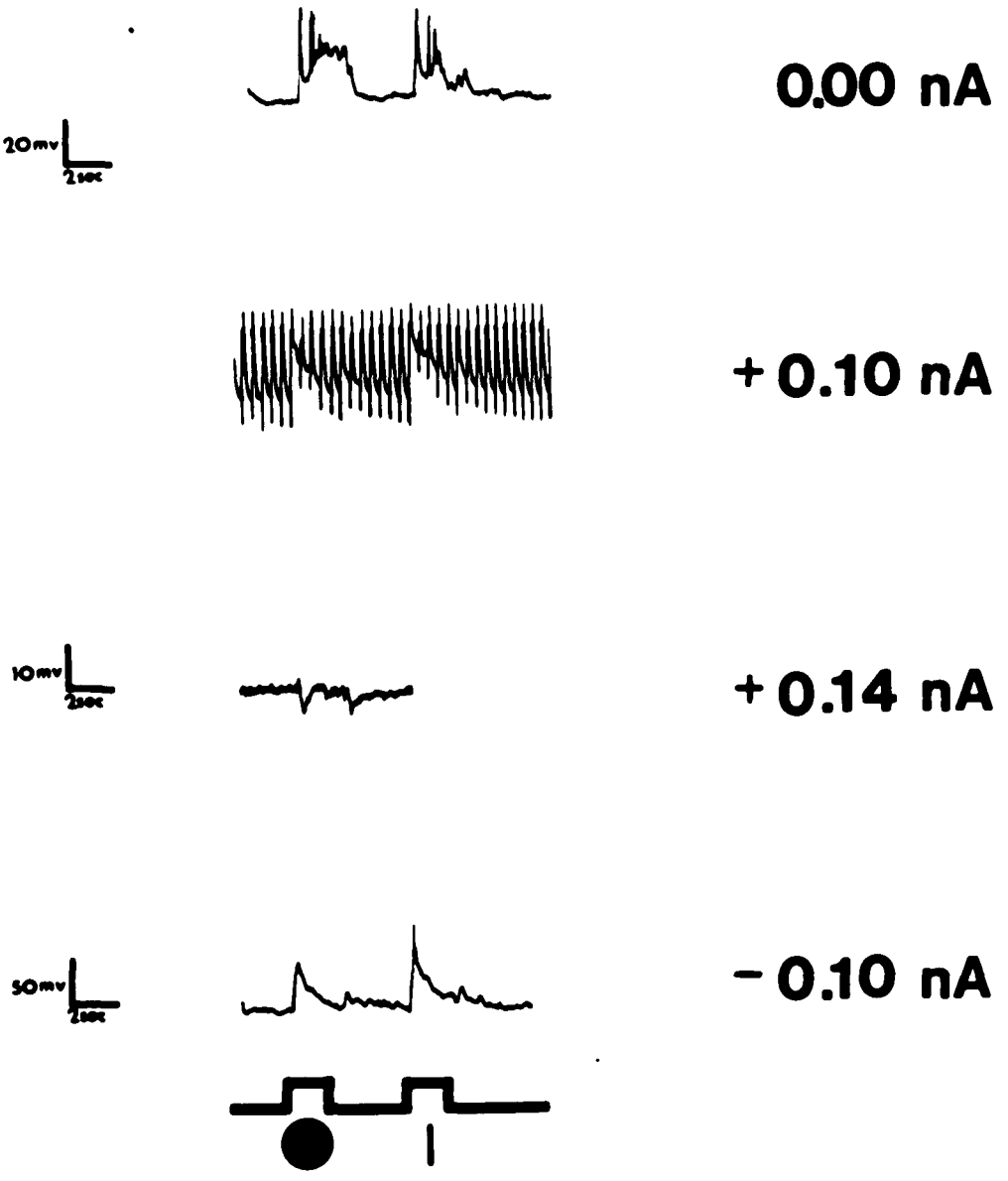

0.00 nA
20mv
2sec
+0.10 nA
10mv
2sec
+0.14 nA
50mv
2sec
-0.10 nA

Figure 34. Intracellular responses from a displaced ON amacrine cell (shown in figures 23a and 25) to diffuse illumination or an 80 um slit (note two illustrated sweep speeds). This cell had a resting potential of -45 mV and a monophasic receptive field. Picrotoxin application attenuated the ON IPSP (upper tracings), while strychnine application (second row of tracings) attenuated the OFF IPSP. In between the application of these antagonists, there was a return to control perfusate (RTC) for over 3 min. The bottom tracings shows that 20 uM APB largely abolished the ON depolarizing response leaving an OFF hyperpolarizing - depolarizing - spike sequence, at light offset. The current injections illustrated in the lower right were obtained in the presence of APB and the dashes show when the stimulus is on.

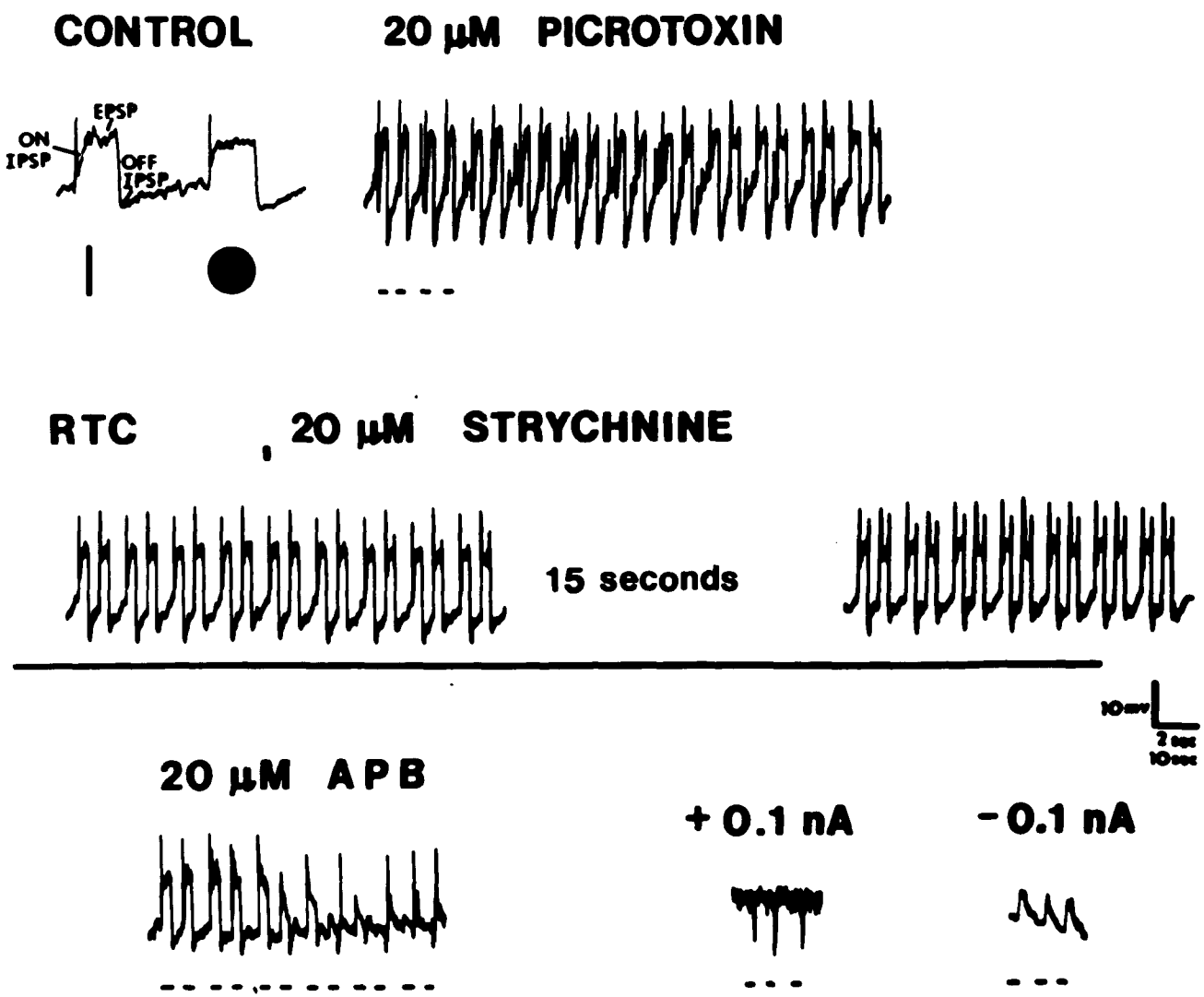
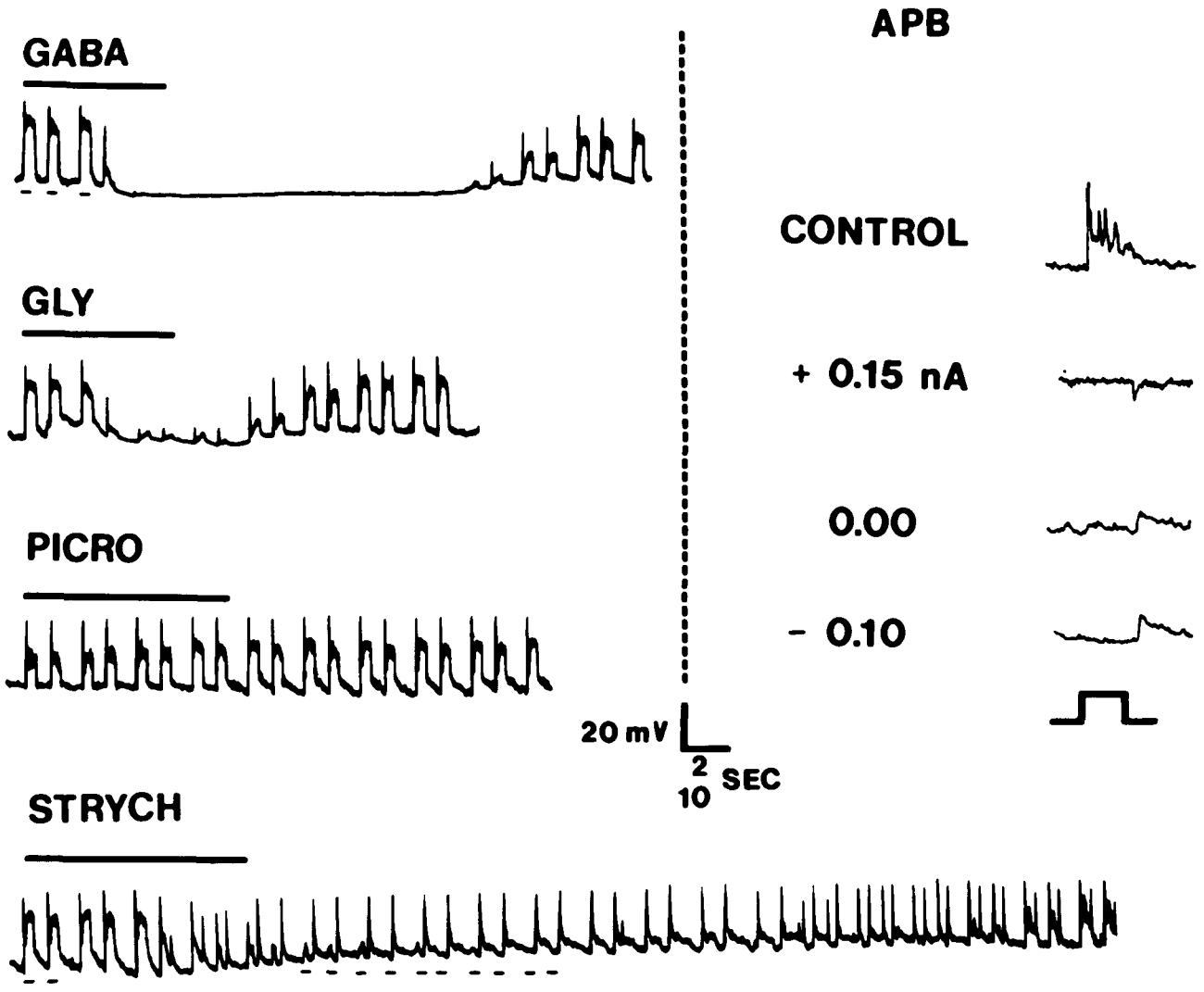

CONTROL
20 µM PICROTOXIN
EPSP
ON IPSP
OFF IPSP
RTC
20 µM STRYCHNINE
15 seconds
10 mV
2 sec
10 sec
20 µM APB
+ 0.1 nA
- 0.1 nA

Figure 35. Intracellular responses from an unstained ON cell (receptive field data shown in figure 25). This cell had a resting potential of -70 mV and a monophasic receptive field. This cell was observed to be more sensitive to the application of 2.5 mM GABA than glycine (upper left two tracings). Twenty five uM picrotoxin enhanced all response component (third tracing left). Twenty five uM strychnine (bottom tracing) depolarized the cell, abolished the ON depolarization and left an OFF depolarization and spike. Fifty uM APB (upper right tracings) was shown to abolish the the ON depolarizing response. The DC current injection experiments shown, on the right, were all obtained while in the presence of APB.


APB
GABA
CONTROL
GLY
+ 0.15 nA
0.00
PICRO
- 0.10
20 mV
2
10 SEC
STRYCH

Chapter VI

General Discussion

The results of the present study can be briefly summarized as follows. First, on the basis of their excitatory response components, morphologically identified amacrine and ganglion cells of the mudpuppy retina can be classified into ON, OFF, and ON-OFF categories. In addition, ON amacrines have been found to have their somas in either the inner nuclear or ganglion cell layers. Collectively, these neurons display a wide variety of morphological features and a functional organization typical of most vertebrates. Second, as also found in many vertebrates, the mudpuppy's retina generally adheres to the principles associated with the functional segregation of dendrites within the inner plexiform layer. Finally, detailed analysis of the post synaptic potentials of third order neurons (ON cells) demonstrates a complex interplay between excitation and inhibition. Some ON cells, the dendrites of which appear to be vitreally stratified, are found to have OFF inhibitory potentials. This indicates that potentials with their genesis in the light or dark are actively involved in the response of a cell. This also indicates that the functional segregation of the ON and OFF systems is not generalizable to inhibitory neurons.

Any classification scheme, for retinal neurons, is based on the ability to form well defined and mutually exclusive categories. These categories must also be sufficiently "spaced" so that the variability in the data does not significantly interfere with the discrimination between them. This problem is most obvious with the sub- classification of ganglion cells. Kolb, Nelson, and Mariani (1981) state that there are 23 types of ganglion cells in cat retina; however, there is considerable difficulty maintaining boundaries between the well established physiologically defined categories of X- and Y- cells (Hochstein and Shapley, 1976). When there are enough morphologically identified ganglion cells to almost have one for every letter of the alphabet, the ablility to physiologically discriminate 2 or 3 groups of ganglion cells (only after the sophisticated analysis) should make one question the the boundaries enforced upon the dependent variables chosen for quantification.

The problem of classification is also present in the discrimination between cell types. Amacrine cells are physically distinguished as they are named -- cells without axons. When a cell is observed to have an axon or its response is recorded in the optic nerve, then this positively identifies the cell as a ganglion cell. All methods of identification of amacrine cells rely on negative evidence. To deal with this situation Naka chooses an alternative classification for the formation of his model (see Appen-

dix). In fact, in an earlier version of his model, Naka (1977) did not even respect the category of ganglion cells. Until there are positive methods of identification of both ganglion and amacrine cells, discriminations must be made cautiously. The present classification of neurons was made in order to conform with existing models that also used the mudpuppy preparation, but must be regarded as tentative. It must be stressed that detailed morphological examination of the origins of intracellular potentials is especially important to the cells of the inner retina. Even though stereotypical responses can be distinguished, the congregate of potentials that comprise the intracellular record of a cell, are often undesirably affected by experimental manipulations. Until intracellular potentials from the vertebrate retina can be regularly recorded, which approach the theoretically expected dimensions, cell classification by post synaptic potentials elicited by photic stimulation *alone* must be regarded as a first approximation.

Organization of Inhibition Within the Inner Retina: Insights Derived from the Study of ON Cells.

Many cells of the inner retina that depolarize and spike only at light onset which also have IPSPs associated with light offset and cannot be explained simply by ON-OFF amacrine cells. As also found by others, the pharmacologi-

cal effects of strychnine selectively block either a transient OFF IPSP that was uninfluenced by picrotoxin, or it blocked the sustained dark release of an inhibitory transmitter in a way similar to Co++ application. Since it is assumed that a cell's transmitter substance is released when it is depolarized, it would then follow that the inhibitory potentials stem from neurons that are depolarized in the dark, a feature associated with the OFF system. The dendrites of the ON and OFF cells were found to demonstrate a degree of separation, which was highly statistically reliable, even though there was overlap between them. Therefore, the interaction between ON and OFF PSPs must be mediated by cells that send their dendrites to the "wrong" sublamina or occurs in the areas of the inner plexiform layer where there is overlap between these two functional groups.

To account for the difference between ON and OFF transient IPSPs, it might be possible that a variety of ON-OFF amacrine cells converge upon a single ganglion cell and that these amacrine cells do not produce uniform IPSPs at light onset and offset. This study and others (Miller, Dacheux and Frumkes, 1977; Chan and Naka, 1980; Vallerga, 1981) have found that ON-OFF amacrine cells are a heterogeneous group. Therefore, a complex hypothesis can be formed involving interactions between inhibitory and/or excitatory transient amacrine cells where the net result may seem to favor light onset or offset. The post synaptic response may be further skewed by additional input from one of the bipo-

lar cell types. Wunk and Werblin (1979) make a similar type of hypothesis to account for the post synaptic potentials observed in their "hybrid" type of ganglion cell.

If the assumption is made that ON and OFF amacrine cells produce transient inhibitory potentials, then the explanation for the independence of ON and OFF IPSPs becomes simpler. This point of view is included in the Miller - Frumkes model (see appendix). Either one of the inhibitory amino acid antagonists used effects both ON and OFF IPSPs in the majority of cases. But on occasion, the ON or the OFF IPSP is attenuated more than the other. In addition, ganglion cells are recorded from in which only an ON IPSP can be detected. The only problem with the inference that ON and OFF amacrine cells produce transient IPSPs is that, the studies that best document the identification of these neurons (of which Naka's are typical) classify them as "sustained" amacrine cells (or type N). Also, they have been hypothesized to be excitatory to a class of large field ganglion cells. This excitatory relationship is inferred by both correlative receptive field data and direct electrical stimulation through type N cells, while simultaneously recording from nearby ganglion cells (Naka, 1977; Davies and Naka, 1980).

As compelling as Naka's evidence might seem, there are some perplexing data that leaves the way open for other conclusions. Unlike the results found in similar stimulation and recording experiments done between bipolar and ganglion

cells, the type N cell is found to drive both ON and OFF ganglion cells. Considering the complex synaptology between amacrine cells, which includes both chemical and electrical synapses, electrical stimulation of these cells may be indirectly effecting ganglion cells. Naka has indicated a modification in the status of type N cells, which were described as being neurons with only sustained potentials, without spikes. He indicates that there are intermittent observations of type N cells with a few spikes. All of the ON and OFF amacrine cells presently recovered had an initial or a terminating action potential, to a pulse of light. Spikes in ON, OFF, and ON-OFF amacrine cells might be the corresponding presynaptic event for the commonly observed transient IPSPs.

To account for the tonic inhibition of ON cells, there are two likely candidates. Irrespective of the transient potentials at light offset, OFF amacrine cells remain depolarized in the dark. Therefore it is possible that they could also be the source of the dark release of an inhibitory amino acid that is blocked by strychnine. Another interesting possibility would be if the glycine accumulating bipolar found in cat by McGuire, Stevens, and Sterling (1980) would be a hyperpolarizing bipolar cell that has its axon terminal in or near the vitreal layer of the inner plexiform layer. All the retinal models under consideration agree that the hyperpolarizing bipolar cells excite OFF ganglion, in addition, the Miller - Frumkes model describes

the mechanism as a disfacilitory one (dark release of excitatory transmitter). Perhaps then ON responding could be assisted by a disinhibitory mechanism mediated by an inhibitory hyperpolarizing bipolar or OFF amacrine cell. This disinhibitory mechanism might be described as a push - pull modulation, because Cl- free (personal observation) and APB disables depolarizing bipolar cell excitation in ON cells which also show evidence of strychnine induced depolarization.

In conclusion, the photic responses of third order retinal neurons can best be understood as a modulation of the ambient level of excitation and inhibition. Certainly, the idea that a response is a modulation of ongoing activity is not new and can be traced back at least as far as the 19th century when Hering and Helmholtz independently postulated the existence of intrinsic light or eigengrau within the eye of the observer. The results of the present study infer a wider variety of mechanisms than were being postulated 10 years ago; however, it is still less than the numbers of neuronal categories currently offered by anatomical and extracellular spike recording studies. Perhaps a study of a much larger population of neurons, with more powerful techniques, will end up with a definite number of distinct ganglion cell types. But hopefully, future research will discern a much smaller number of mechanistic principles which will make such numerical accounts superfluous.

APPENDIX

I. Naka's Model (Naka, 1976, 1977; Chan and Naka, 1980)

Naka's schema of the outer plexiform layer involves the usual receptor, horizontal cell and bipolar cell interactions. Moreover, he provided direct evidence demonstrating that the depolarizing bipolar cell is the source of ON excitation while the hyperpolarizing bipolar cell is the source for OFF, in ganglion cells. In contrast, Naka maintains a unique organization of the inner plexiform layer. He does not fit his classes of inner retinal neurons into the usual amacrine - ganglion cell dichotomy based on morphological characteristics alone. Instead, he groups cells into categories of his own making that are primarily physiological. To accomplish this, Naka employs a method of "noise" analysis which utilizes measurements of a cell's response to a mathematically specified input, whether it be electrical or photic. Through the mathematical rigor of the non - linear analysis of Weiner (Marmarelis and Naka, 1973), a cell's response to many spatial and temporal frequencies (in addition to the interaction between frequencies) can be quantified. The result of this methodology, called a kernel, is the synthesis of a wide range of expected responses in either the space or time domain. It is these kernels that are major basis of comparison. Simply stated, the major distinctions between functional types is the proportion

of the cell's response that is accounted for by the first order (linear) vs. higher order (nonlinear) kernels and monophasic (homogeneous) vs. biphasic (having center - surround antagonism) receptive fields.

Naka maintains that many ganglion (type Y cells) receive their excitatory input from bipolar cells. This is supported by electrical and photic stimulation experiments (Naka, 1976; Davies and Naka, 1980). In a more recent version of his model, ganglion cells with larger dendritic fields and receptive fields are accommodated. But, these larger ganglion cells have different receptive fields than bipolar cells; therefore Naka concludes that their source of excitation is not the bipolars (Davies and Naka, 1980).

Naka correlates the excitatory PSPs of the larger ganglion cells with a class of cells that are usually found in the area of the inner nuclear layer where amacrine cells are found. He refers to these cells with sustained graded potentials as type N cells. Others have referred to similar cells as sustained ON or OFF amacrine cells (Kaneko, 1970). Naka also describes another inner nuclear layer cell, with transient depolarizations at light onset and offset. This is his type C which has been hypothesized to be a displaced ganglion cell (Naka, 1976a) or later on, determined the possibility that it may be an ON-OFF amacrine cell (Chan and Naka, 1981). Naka is accumulating evidence that these type N and C cells receive their excitatory input from horizontal cells, and not from the bipolars (Naka, 1982). In addition

to his unique categorization of cells, Naka's model of function is organized using the logical permutations of sign preserving and/or inverting synapses. This avoids the controversies concerning the role of the physiological mechanisms of excitation and inhibition (Naka, 1976b).

II. Werblin's Model (Wunk and Werblin, 1979; Werblin, 1979)

The strategy that Werblin follows is to analyze changes in a cell's PSP utilizing alterations of stimulus parameters such as: intensity, size, and time. Werblin assumes that there must be stimuli that are optimal for a given class of neurons and hence, a maximized post synaptic potential. In addition through the use of current injection to demonstrate direct control over the post synaptic potentials and exaggerate their appearance, the ionic basis of the response can be inferred and attributed to various presynaptic events.

The most successful finding from this method has been in the contrasting of responses elicited by a small spot centered over the neuron's perikarya vs. the stimulation of the surrounding area. A small spot, less than 400 μm is optimal for eliciting the excitatory influences from the bipolar cells of mudpuppy and therefore causes the post synaptic ganglion cell to responds maximally. Upon stimulation of the surround, the polarity of bipolars is antagonized and the output of the post synaptic ganglion cell is modified. This activity occurs in the presence of a steady surround.

Werblin maintains that the center - surround antagonism in ganglion cells is the result of the modification of the bipolar cell response by the horizontal cell.

Unlike Naka, Werblin deals directly with inhibitory potentials in ganglion cells. He hypothesizes that amacrine cells are source of transient inhibition and this type of inhibition is not involved in center - surround antagonism. Werblin has developed photic stimulation techniques to support the hypothesis that ganglion cells are inhibited by amacrine cells. He demonstrated that if a ganglion cell's surround is stimulated with a rotating annulus of discontinuous sectors, to simulate the action of moving vanes on a windmill, it has little effect on the mechanisms of center - surround antagonism, but will tonically hyperpolarize many ON, OFF or an ON-OFF ganglion cell (Werblin and Copenhagen, 1974). Correlated with this ganglion cell inhibition is the steady depolarization of the ON-OFF amacrine cells by the same moving windmill. Werblin has demonstrated that this relationship holds over a limited range of intensities and angular velocities. Werblin also demonstrates that horizontal, amacrine, and ganglion cells all respond to a stepwise increase in intensity of the stationary windmill; but the sustained post synaptic potentials, just mentioned, maximally occur with movement of the already present windmill. The horizontal cell in the same situation is virtually unaffected (Thibos and Werblin, 1978).

Werblin adds that all ganglion cells are inhibited in a

transient manner by the ON-OFF amacrine cell, which is the only one he recognizes in mudpuppy and tiger salamaner. Werblin maintains that ON-OFF ganglion cells don't receive direct excitatory bipolar cell input. As discussed in chapter III, ON-OFF ganglion cells have restricted, central excitatory areas. To accommodate this empirical finding, Werblin hypothesizes the existence of an excitatory interneuron that's responds maximally to small stimuli at light onset and offset. This hypothetical neuron, which has never been recorded from or identified in any way, is called a Narrow Field Transient. Werblin distinguishes a second type of ON-OFF ganglion cell, the Hybrid. This cell has a much larger OFF excitatory postsynaptic potential than ON. Also, in response to surround stimulation a sustained depolarization occurs. The Hybrid cell has excitatory input from both the Narrow field transient and the hyperpolarizing bipolar cell. In summary, Werblin's model of the inner plexiform layer includes the inhibitory activity of amacrine cells, in addition to the excitatory influence of the bipolars. Werblin maintains that both of his classes of ON-OFF ganglion cells are excited by a hypothetical, unidentified cell the Narrow Filed Transient. This, as yet unrecorded from cell, would account for ganglion cell excitation in those cells not directly connected to bipolars (Wunk and Werblin, 1979). Vallerga (1981) has identified three types of ON-OFF amacrine cells in the retina of the tiger salamander, of which two are restricted to sublamina b of the inner plexi-

form layer. In a model similar to Werblin's, she maintains that these narrowly stratified amacrine are driven by a multistratified, excitatory ON-OFF amacrine cell.

Miller and Frumkes' Model (Miller and Dacheux, 1976c; Miller, 1979; Frumkes, et al., 1981)

The model of the inner plexiform layer that Miller and Frumkes proposes (Frumkes, et al., 1981) came out of their physiological studies of the inhibitory influences upon ganglion cells. This work has primarily used the superfused preparation of the mudpuppy retina. Inhibition has been studied by changing the extracellular environment by altering the ionic balance, as with the chloride free experiments (Miller and Daucheux, 1976b) and then with the addition of various neurotransmitters that have been demonstrated to be inhibitory. These include: GABA and glycine (Frumkes, et al., 1981), taurine (Cunningham and Miller, 1980) and serine and alanine (Slaughter and Miller, 1982b).

As a general overview, Miller maintains that all ganglion cell responses reflect interactions between amacrine cell inhibition and bipolar cell excitation. This model takes the parsimonious position that the variation in ganglion cell behavior is due to the degree of amacrine cell involvement, rather than postulated hypothetical neurons or connections. The Miller and Frumkes model supports the excitatory role of bipolars on ganglion cells, but unlike

Werblin, maintain that bipolars also directly excite ON-OFF ganglion cells. Differences between classes of neurons can be accounted for by unequally weighted inputs that converge upon them. In other words, subcatagories of ganglion cells were developed according to the magnitude of their separate presynaptic influences. Like Werblin, Miller maintains that most ganglion cells receive transient ON-OFF inhibition and that its genesis is usually an inhibitory amino acid releasing ON-OFF amacrine cell. But because there is evidence for the independence between ON and OFF inhibitory post synaptic potentials, Miller and Frumkes work into their model ON and OFF amacrine cells as inhibitory interneurons. The evidence here is that GABA and its antagonists have a greater influence on depolarizing than hyperpolarizing bipolar cells, while glycine and its antagonists has a greater influence on hyperpolarizing than depolarizing bipolar cells. In addition, some ON IPSPs are more sensitive to GABA - picrotoxin effects, where some OFF IPSPs are more sensitive to glycine - strychnine effects. (Frumkes, et al., 1981).

The following summary table outlines the three models:

INFLUENCES IN IPL	NAKA	WERBLIN	MILLER & FRUMKES
SOURCE OF EXCITATION IN ON, OFF GANGLION CELLS	bipolars, type N cells	bipolars,	bipolars,
CENTER-SURROUND ANTAGONISM IN ON, OFF GANGLION CELLS	modulation of bipolars by horizontal cells	same	same
SOURCE OF EXCITATION IN ON-OFF GANGLION CELLS	horizontal cell, type C cells	narrow field transient	bipolars
SOURCE OF EXCITATION IN AMACRINE & TYPE N CELLS	horizontal cells	bipolars	bipolars
SOURCE OF INHIBITION	sign inverting synapses	ON-OFF amacrine cells	ON, OFF, ON-OFF amacrine cells

All three models agree that the two bipolar types constitute the major excitatory input to some ganglion cells. In other respects, however, each paints a different functional picture of the inner retina.

Bibliography

Barlow, H. B. Sumation and inhibiton in the frog's retina. Journal of Physiology, 1953, 119, 69-88.

Baylor, D. A., & Fuortes, M. G. F. Electrical responses of single cones in the retina of the turtle. Journal of Physiology, 1970, 207, 77-92.

Belgum, J. H., Dvorak, D. R., & McReynolds, J. S. Center stimuli elicit transient and sustained IPSPs in mudpuppy retinal ganglion cells. (Abstract), Society for Neuronscience, 1980, Meeting. Cincinnati, Ohio, p. 212.

Belgum, J. H., Dvorak, D. R., & McReynolds, J. S. Sustained synaptic input to ganglion cells of mudpuppy retina. Journal of Physiology, 1982, 326, 91-108.

Bloomfield, S. A. A physiological - morphological study of neuronal pathways in the rabbit retina. (Ph. D. Thesis), Washington University, Saint Louis, Missouri, 1981.

Boycott, B. B., & Dowling, J. E. Organization of the primate retina: light microscopy. Proceedings of the Royal Society B, 1969, 255, 109-184.

Boycott, B. B., & Kolb, H. The connections between bipolar cells and photoreceptors of the domestic cat. Journal of Comparative Neurology, 1973, 148, 91-114.

Boycott, B. B., & Wassle, H. The morphological types of ganglion cells of the domestic cat's retina. Journal of Physiology, 1974, 240, 397-419.

Brown, K. T., & Wiesel, T. N. Intraretinal recording with micropipette electrodes in the intact cat eye. Journal of Physiology, 1961, 149, 537-562.

Budge, B. 3-D Graphics System and Game Tool, (Computer Program) California Pacific Computer Co., 1980.

Cajal, S. R. [La retine des vertebres] In Rodieck, R. W. The Vertebrate Retina, W. H. Freeman and Company, San Francisco. (translated from La Celle, 1893, 9, 17-257.)

Caldwell, J. H., & Daw, N. W. Effects of picrotoxin and strychnine on rabbit retinal ganglion cells: change in center surround receptive fields. Journal of Physiology, 1978, 276, 298-310.

Caldwell, J. H., Daw, N. W., & Wyatt, H. J. Effects of picrotoxin and strychnine on rabbit retinal ganglion cells: lateral interactions for cells with more complex receptive fields. Journal of Physiology, 1978, 276, 277-298.

Cleland, B. G., & Levick, W. R. Brisk and sluggish concentrically organized ganglion cells in the cat's retina. Journal of Physiology, 1974, 240, 421-456.

Cleland, B. G., Levick, W. R., & Wassle, H. Physiological identification of a morphological class of ganglion cell. Journal of Physiology, 1975, 248, 151-171.

Conn, H. J., Darrow, M. A., & Emmel, V. M. (Eds.) Staining Procedures: Biological Stain Commission, The Williams & Wilkins Company, Baltimore, 1962.

Cunningham, R., & Miller, R. F. Taurine: its selective action on the rabbit. Brain Research, 1976, 117, 341-345.

Dacheux, R. F. Connections of the small bipolar cells with the photoreceptors in the turtle. An electron microscope study of Golgi-impregnated, gold-toned retinas. Journal of Comparative Neurology, 1982, 205, 55-62.

Dacheux, R. F., Frumkes, T. E., & Miller, R. F. Pathways and polarities of synaptic interactions in the inner retina of the mudpuppy. I. Synaptic blocking studies. Brain Research, 1979, 161, 1-12.

Davies, G. W., & Naka, K. I. Spatial organizations of catfish retinal neurons. Journal of Neurophysiology, 1980, 43, 807-831.

Dowling, J. E. Information processing by local circuits: the vertebrate retina as a model system. The Neurosciences, MIT Press, (eds. Schmitt, F. O., & Worden, F. G), 1979.

Dowling, J. E., & Boycott, B. B. Organization of the primate retina: Electron microscopy. Proceedings of the Royal Society B, 1966, 170, 80-111.

Dowling, J. E., & Werblin, F. S. Organization of retina of the mudpuppy, Necturus maculosus. I. Synaptic structure. Journal of Neurophysiology, 1969, 23, 315-338.

Enroth-Cugell, C., & Robson, J. G. The contrast sensitivity of retinal ganglion cells of the cat. Journal of

Physiology, 1966, 187, 517-522.

Famiglietti, E. V., Kaneko, A., & Tachibana, M. Neuronal architecture of on and off pathways to ganglion cells in carp retina. Science, 1977, 198, 1267-1269.

Famiglietti, E. V., & Kolb, H. Structural basis for ON- and OFF- center responses in retinal ganglion cells. Science, 1976, 8, 193-195.

Fleshman, J. W., Burke, R. E., Glenn, L. L., Lev-Tov, A., & Miller, J. P. Cell size and specific membrane resisitivity of type-identified cat spinal motoneurons: morphological, physiological and modeling studies. (Abstract), Society for Neuroscience, 1982, Meeting. Minneapolis, Minnesota, p. 414.

Friedlander, M. J., Lin, C. S., Stanford, L. R., & Sherman, S. M. Morphology of functionally identified neurons in lateral geniculate nucleus of the cat. Journal of Neurophysiology, 1981, 46, 80-129.

Frumkes, T. E., Coleman, P. A., and Nicotera, N. Discfacilitation and inhibition in the vertebrate retina. (Abstract), Society for Neuronscience, 1979, Meeting. Atlanta, Georgia, p. 786.

Frumkes, T. E., Coleman, P. A., and Nicotera, N. Inhibitory input to amphibian retinal ganglion cells. (Abstract), Association for Research in Vision and Opthalamology. 1980, Meeting. Orlando, Flordia, p. 130.

Frumkes, T. E., & Miller, R. F. Pathways and polarities of synaptic interactions in the inner retina of the mudpuppy: II. Insight revealed by an analysis of latency and threshold. Brain Research, 1979, 161, 13-24.

Frumkes, T. E., Miller, R. F., Slaughter, M., & Dacheux, R. F. Physiological and pharmacological basis of GABA and glycine action on neurons of mudpuppy retina. III. Amacrine- mediated inhibitory influences on ganglion cell receptive - field organization: a Model. Journal of Neurophysiology, 1981, 45, 783-803.

Guillery, R. W. A study of Golgi preparations from the dorsal lateral geniculate nucleus of the adult cat. Journal of Comparative Neurology, 1966, 238, 21-50.

Hartline, H. K. The response of single optic nerve fiers of the vertebrate eye to illumination of the retina. American Journal of Physiology, 1938, 121, 400-415.

Hochstein, S., & Shapley, R. M. Quantitative analysis of retinal ganglion cell classifications. Journal of Physiology, 1976, 262, 237-264.

Hubel, D. H., & Wiesel, T. N. Receptive fields and functional architecture of monkey striate cortex. Journal of Physiology, 1968, 195, 215-243.

Ikeda, H., & Sheardown, M. Functional transmitters for retinal ganglion cells in the cat. (Abstract), The Association for Research in Vision and Ophthalmology, 1982, Meeting. Sarasota, Florida, p. 175.

Jahnsen, H., & Llinas, R. Electrophysiological properties of guinea pig thalamic neurons studied in vitro. (Abstract), Society for Neuroscience, 1982, Meeting. Minneapolis, Minnesota, p. 413.

Junge, D. Nerve and Muscle Excitation, Sinauer Associates, Inc., Sunderland, Massachusetts, 1981.

Kaneko, A. Physiological and morphological identification of horizontal, bipolar and amacrine cells in goldfish retina. Journal of Physiology, 1970, 207, 623-633.

Karwoski, C. J., & Burkhardt, D. A. Ganglion cell responses of the mudpuppy retina to flashing and moving stimuli. Vision Research, 1976, 16, 1483-1495.

Kiernan, J. A. Histological & Histochemical Methods: Theory & Practice, Pergamon Press, Oxford, 1981.

Kirby, A. W., & Enroth-Cugell, C. The involvement of gamma-amino butyric acid in the organization of cat retina ganglion cell receptive fields. A study with picrotoxin and bicuculline. Journal of General Physiology, 1976, 68, 465-484.

Kirby, A. W., & Schweitzer-Tong, D. E. GABA antagonists alter spatial summation in receptive field centers of rod but not cone driven cat retinal ganglion Y-cells. (Abstract), Association for Research in Vision and Ophthalmology, 1980, Meeting. Orlando, Florida, 132.

Kolb, H., & Famiglietti, E. V. Rod and cone pathways in the inner plexiform layer of cat retina. Science, 1981, 186, 47-49.

Kolb, H., & Nelson, R. Amacrine cells of the cat retina. Vision Research, 1981, 21, 1625-1634.

Kolb, H., Nelson, R, & Mariani, A. Amacrine cells, bipolar cells, and ganglion cells of the cat retina: a Golgi

study. Vision Research, 1981, 21, 1081-1114.

Kuffler, S. W. Discharge patterns and functional organization of mammalian retina. Journal of Neurophysiology, 1953, 16, 37-68.

Lasansky, A. Lateral contacts and interactions of horizontal cell dendrites in the retina of the larval tiger salamander. Journal of Physiology, 1980, 301, 59-68.

Lennie, P. Parallel visual pathways: a review. Vision Research, 1980, 20, 561-594.

Marc, R. E., Lam, D. M. K. Glycinergic pathways in the goldfish retina. Journal of Neurosciences, 1981, 1, 152-165.

Marc, R. E., Stell, W. K., Bok, D., & Lam, D. M. K. GABAergic pathways in the goldfish retina. Journal of Comparative Neurology, 1978, 182, 221-245.

Marchiafava, P. L. The responses of retinal ganglion cells to stationary and moving stimuli. Vision Research, 1979, 19, 1203-1211.

Marmarelis, P. Z., & Naka, K. I. Nonlinear analysis and synthesis of receptive-field responses in the catfish retina. Journal of Neurophysiology, 1973, 36, 605-618.

Marr, D. Vision W. H. Freeman and Company, San Francisco, 1982.

McGuire, B. A., Stevens, J. K., & Sterling, P. Beta ganglion cells recieve convergent input from 2 types of cone bipolars. (Abstract), Society for Neuronscience, 1980, Meeting. Cincinnati, Ohio, p. 347.

Miller, J. P., & Rall, W. Effect of dendritic length upon synaptic efficacy. (Abstract), Society for Neurosience, 1982, Meeting. Minneapolis, Minnesota, p. 414.

Miller, R. F. The neuronal basis of ganglion-cell receptive-field organization and the physiology of amacrine cells. The Neurosciences, MIT Press, (eds. Schmitt, F. O., & Worden, F. G), 1979.

Miller, R. F. & Dacheux, R. F. Synaptic organization and ionic basis of on and off channels in mudpyppy retina. III. A model of ganglion cell receptive field organization based on chloride - free experiments. Journal of General Physiology, 1976, 67, 679-690.

Miller, R. F., Dacheux, R. F. Intracellular chloride activity in retinal neurons. (Abstract), The Association for Research in Vision and Ophthalmology, 1976, Meeting. Sarasota, Florida, p. 29.

Miller, R. F., & Dacheux, R. F. Dendritic and somatic spikes in mudpuppy amacrine cells: identification and TTX sensitivity. Brain Research, 1976, 104, 157-162.

Miller, R. F., Dacheux, R. F., & Frumkes, T. E. Amacrine cells in necturus retina: Evidence for indpepndent gamma - aminobutyric acid- and glycine - releasing neurons. Science, 1977, 18, 748-750.

Miller, R. F., Frumkes, T. E., Slaughter, M., & Dacheux, R. F. Physiological and pharmacological basis of GABA and glycine action on neurons of mudpuppy retina. I. Receptors, horizontal cells, bipolars, and G- cells. Journal of Neurophysiology, 1981, 45, 743-763.

Miller, R. F., Frumkes, T. E., Slaughter, M., & Dacheux, R. F. Physiological and pharmacological basis of GABA and glycine action on neurons of mudpuppy retina. II. Amacrine and ganglion cells. Journal of Neurophysiology, 1981, 45, 764-781.

Mori, K., Nowycky, M. C., & Shepherd, G. M. Electrophysiological analysis of mitral cells in the isolated turtle olfactory bulb. Journal of Physiology, 1981, 314, 281-294.

Naka, K. I. Neuronal circuitry in the catfish retina. Investigative Opthalmology, 1976, 15, 926-935.

Naka, K. I. Functional organization of catfish retina. Journal of Neurophysiology, 1977, 40, 26-43.

Naka, K. I. The cells horizontal cells talk to. Vision Research, 1982, 22, 653-660.

Naka, K. I., & Christensen, B. N. Direct electrical connections between transient amacrine cells in the catfish retina. Science, 1981, 214, 462-464.

Naka, K. I., & Ohtsuka, T. Morphological and functional identifications of catfish retinal neurons. II. Morphological identification. Journal of Neurophysiology, 1975, 38, 72-91.

Nelson, R. AII amacrine cells quicken time course of rod signals in the cat retina. Journal of Neurophysiology, 1982, 47, 928-947.

Nelson, R., Famiglietti, E. V., & Kolb, H. Intracellular staining reveals different levels of stratification for on- and off- center ganglion cells in the cat retina. Journal of Neurophysiology, 1978, 41, 472-483.

Olsen, J. S., & Miller, R. F. Spontaneous slow potentials and spreading depression in amphibian retina. Journal of Neurophysiology, 1977, 40, 752-767.

Parnas, I. Propagation in nonuniform neurites: form and function in axons. The Neurosciences, MIT Press, (eds. Schmitt, F. O., & Worden, F. G), 1979.

Polyak, S. L. The Retina, Chicago, Chicago University Press, 1941.

Rall, W. Dendritic neuron theory and dendrodendritic synapses in a simple cortical system. The Neurosciences: Second Study Program (F. O. Schmidt, ed.-in-chief). New York: Rockefeller. 1970, pp. 552-565.

Raviola, E., & Gilula, N. B. Intramembrane organization of specialized contacts in the outer plexiform layer of the retina. A freeze-fracture study in monkeys and rabbits. Journal of Cell Biology, 1975, 65, 192-222.

Rodieck, R. W. The Vertebrate Retina, W. H. Freeman and Company, San Francisco, 1973.

Schwartz, E. A. Responses of bipolar cells in the retina of the turtle. Journal of Physiology, 1974, 236, 211-224.

Slaughter, M. M., & Miller, R. F. 2- amino- 4- phosphonobutyric acid: A new pharmacological tool for retinal research. Science, 1981, 211, 182-185.

Slaughter, M., & Miller, R. F. An excitatory amino acid antagonist blocks cone input to sign-conserving second-order retinal neurons. Science, 1983, 219, 1230-1233.

Stone, J. The Whole Mount Handbook, Maitland Publications Pty. Ltd. 1981.

Stone, J., Dreher, B., & Leventhal, A. Hierarchical and parallel mechanisms in the organization of visual cortex. Brain Research Reviews, 1979, 1, 345-394.

Stone, J., & Hoffman, K. -P. Very slow conduction ganglion cells in the cat's retina: A manor new functional type? Brain Research, 1972, 43, 610-616.

Turner, D. A., & Schwartzkroin, P. A. Steady-state electrotonic analysis of intracellularly stained hoppocampal neurons. Journal of Neurophysiology, 1980, 44, 184-199.

Vallerga, S. Physiological and morphological identification of amacrine cells in the retina of the larval tiger salamander. Vision Research, 1981, 21, 1307-1317.

Wassle, H., Levick, W. R., & Cleland, B. G. The distribution of the alpha type of ganglion cells in the cat's retina. Journal of Comparative Neurology, 1975, 159, 419-438.

Weiler, R., & Machiafava, P. L. Physiological and morphological study of the inner plexiform layer of the turtle retina. Vision Research, 1981, 21, 1635-1638.

Werblin, F. S. Response of retinal cells to moving spots: intracellular recording in necturus maculosus. Journal of Neurophysiology, 1970, 32, 342-350.

Werblin, F. S. Regenerative amacrine cell depolarization and formation of on-off ganglion cell responses. Journal of Physiology, 1977, 264, 767-785.

Werblin, F. S. Integrative pathways in local circuits between slow-potential cells in the retina. The Neurosciences, MIT Press, (eds. Schmitt, F. O., & Worden, F. G), 1979.

Werblin, F. S., & Copenhagen, D. R. Control of retinal sensitivity III. Lateral interactions at the inner plixiform layer. The Journal of General Physiology, 1974, 63, 88-110.

Werblin, F. S., & Dowling, J. E. Organization of retina of the mudpuppy, Necturus maculosus. II. Intracellular recording. Journal of Neurophysiology, 1969, 23, 339-355.

Wiesel, T. N. Receptive fields of ganglion cells in the cat's retina. Journal of Physiology, 1960, 153, 583-594.

Wong-Riley, M. T. T. Synaptic organization of the inner plexiform layer in the retina of the tiger salamander. Journal of Neurocytology, 1974, 3, 1-33.

Wunk, D. F., & Werblin, F. S. Synaptic inputs to the ganglion cells in the tiger salamander retina. The Journal of General Physiology, 1979, 73, 265-286.

Yazulla, S. GABAergic synapses in the goldfish retina: an autoradiographic study of 3H-muscimol and 3H-GABA binding. Journal of Comparative Neurology, 1981, 200, 83-93.

Zimmerman, R. P., & Dowling, J. E. Functional stratification of the IPL of the mudpuppy retina. Investigative Ophthalmology and Visual Science Supplement, 1979, p. 35.

Zrenner, E., Nelson, R., & Mariani, A. Intracellular recordings from a biplexiform cell in Macaque retina. (Abstract), The Association for Research in Vision and Ophthalmology, 1982, Meeting. Sarasota, Florida, p. 279.