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A Descriptive Catalog of Dimming Cells in the Purple Shore Crab HEMIGRAPSUS NUDUS

Eugene Pak

ABSTRACT

A DESCRIPTIVE CATALOG OF DIMMING CELLS
IN THE PURPLE SHORE CRAB HEMIGRAPSUS NUDUS

by Eugene Pak

A population of cells responsive to the decrease or dimming of light was identified in the Purple shore crab *Hemigrapsus nudus*. Extracellular pin electrode recordings from the optic nerve tract measured responses to decremental changes in relative light levels over the range 40 to 0.08 lux. The observed activity revealed that these dimming cells could respond over this entire range. A catalog detailing the response characteristics for each of the thirty dimming cells in the study was generated. The parameters measured the receptive field size; response to mechanoreceptive tactile stimuli; response to abrupt On or Off light level changes; response to object movement; and any changes in the dimming activity.

The results indicate that *Hemigrapsus nudus* dimming cells comprise a number of response subgroups, in addition to the observed dimming response. The majority of dimming cells (n=20) shared a whole eye receptive field, and all cells detected small changes or jitter in an object's spatial position. Dimming cells were found to respond to light termination only (n=12), or to both light termination and onset (n=9); no cells had a greater response for light onset than for its termination. The study population suggested that dimming cells had multimodal properties, since all cells responded to both tactile and visual stimuli. There also is evidence that

some cells had regions of increased sensitivity to certain light levels that were more prominent than for the rest of its response range. Dimming cells with enhanced ranges were separated based on the extent of the increased sensitivity into Brief Range (BR) and Extended Range (ER) responses. BR cells had enhanced responses at very specific light levels; ER cells responded over a portion of their total response range.

Dimming cell responses increased in number with decreasing light levels for almost all response groups. The only exception was for dimming cells responding to light Off only; these cells maintained a constant population (n=12) over all light levels measured. Dimming cells appear important for object movement detection rather than for feature perception in low light environments.

LOMA LINDA UNIVERSITY
Graduate School

A DESCRIPTIVE CATALOG OF DIMMING CELLS IN THE PURPLE SHORE CRAB HEMIGRAPSUS NUDUS

by
Eugene Pak

A Thesis in Partial Fulfillment
of the Requirements for the Degree
Master of Science in Biology

June 1988

Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

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INTRODUCTION

Crustacea, and in particular decapod crustaceans such as crabs, crayfish, and lobsters, have served in the study of visual information processing. These crustacea share a common visual organization: a compound eye situated at the end of an eyestalk. Besides allowing for a wide degree of rotation, the eyestalk also encloses the neural elements related to vision. Typically, the ommatidial photoreceptors cover the distal or terminal, surface of the eyestalk; within the distal eyestalk segment itself are four optic ganglia-- the lamina ganglionaris, medulla externa, medulla intermedialis, and medulla terminalis. The proximal segment supports the distal segment, and provides the muscle attachment sites necessary for eyestalk movement. The optic nerve tract passes through these eyestalk segments, connecting the optic ganglia to the supraesophageal ganglia of the crustacean protocerebrum (Figure 1). The optic nerve is best described as a nerve tract, since it carries both descending afferent visual interneurons and ascending efferent motor interneurons.

The study of visual responses in crustaceans developed from research on the crayfish central nervous system. Recordings taken by Waterman and Wiersma (1954,1963), and by Wiersma, Ripley and Christensen (1955) from esophageal commissural interneurons indicated that some of these interneurons carried visual signals. Wiersma and Mill (1965) concluded that the visually responding interneurons in the crayfish commissures responded to three modes of stimulation: movement within a given receptive field; changes in relative illumination levels; and changes in total illumination levels. Despite the relatively simple neural organization of

Figure 1. Diagrammatic representation of *Hemigrapsus nudus*. An enlargement of the right eyestalk with the underlying optic neural ganglia is projected over the body in the drawing. The five shaded regions represent the neural structures related to visual processing. No attempt is made to show interneuron connections between these ganglia, or with the optic tract fibers leading to the central supraesophageal ganglion (not shown).

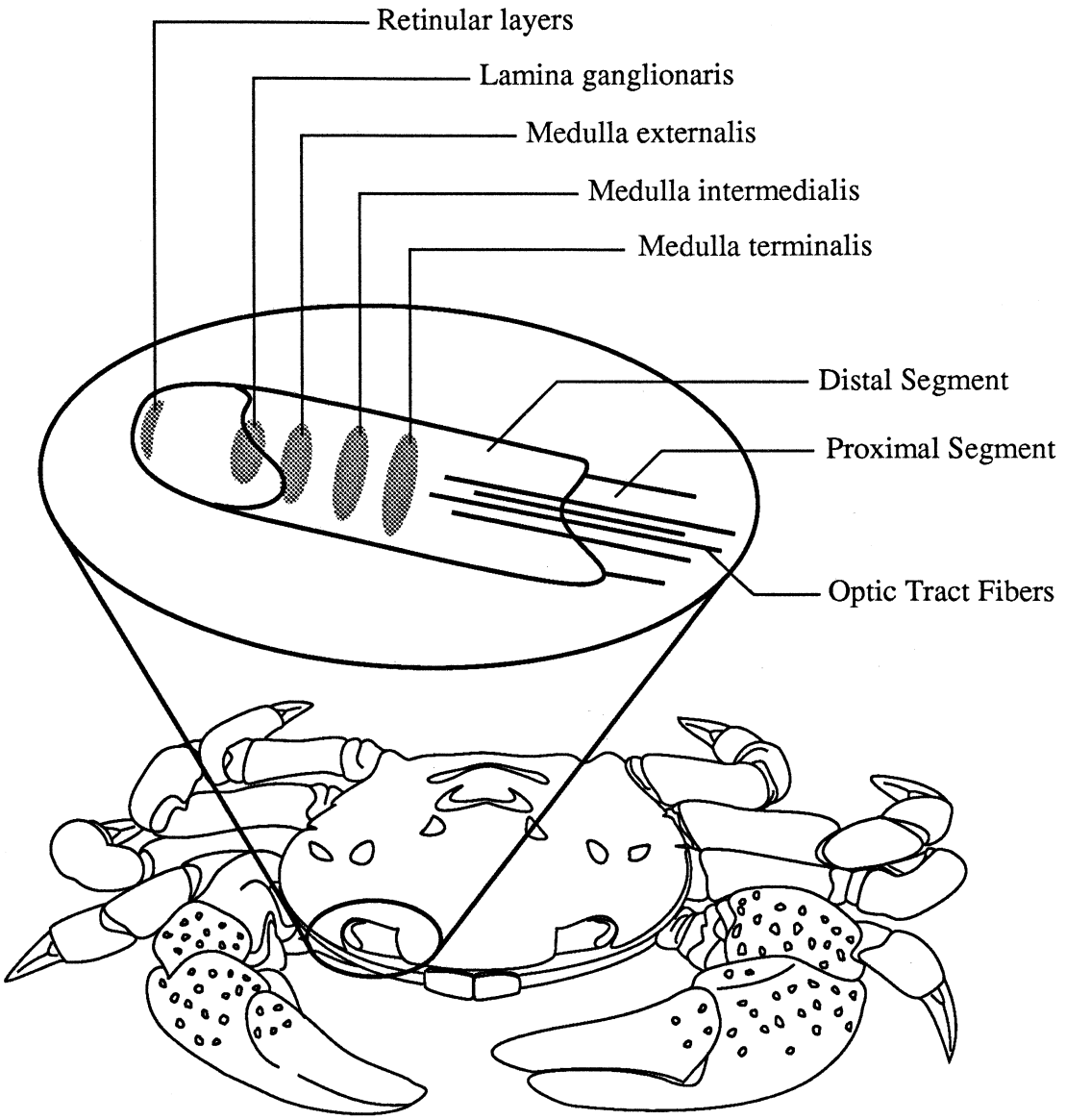

Retinular layers
Lamina ganglionaris
Medulla externalis
Medulla intermedialis
Medulla terminalis
Distal Segment
Proximal Segment
Optic Tract Fibers

Figure 1.

this visual system, it has been difficult to correlate behavioral responses to the anatomical structures. The optic ganglia, with their connecting interganglion fibers, have been difficult to study directly since the eyestalk carapace is difficult to penetrate without disturbing the underlying neural elements. Instead, recordings are taken below these optic ganglia, at the level of the optic nerve tract. Secondary and tertiary responses to visual stimuli have been correlated to afferent visual interneuron responses observed in this optic tract. The method of random probing has provided detailed and reproducible observations concerning crustacean visual processing.

Allowing for certain morphological differences between lobsters, crayfish, and crabs, decapod crustaceans share functional physiological responses. Certain characteristic properties of crustacean vision have been established. These responses correspond to different classes of visual interneurons; indeed York and Wiersma (1975) catalog fourteen separate types of distinct visual responses, not including various optokinetic and otherwise mechanoreceptive responses. Each of these visual responses is defined by a particular receptive field. A receptive field represents that topographic region of the total ommatidial surface which is stimulated by a given stimulus. These receptive fields serve as markers for a certain stimulus and its response. Wiersma and Yamaguchi (1966), for example, identified crayfish optic fiber O38 as a homolateral sustaining fiber, whose receptive field is at the upper back rim of the eye. Optic fiber O38 identifies then a specific interneuron fiber with a characteristic receptive field and response.

These visual fibers respond to a variety of signals, ranging from object movement, spatial-horizon orientation, and to changes in light intensities. The two sets of fibers that respond to changing illumination levels are the sustaining fibers and dimming fibers. Both fiber types have action potential frequencies that are proportional to the amount of dimming. Sustaining fibers increase their discharge rate to increasing light intensity; dimming fibers, however, increase their discharge rates to decreased illumination.

Wiersma (1966) noted that dimming fibers are clearly separate from "OFF" fibers and certain movement fibers which can respond to lowered light levels. Even though Waldrop and Glantz (1985) chose to describe dimming fibers as "tonic OFF optic tract interneurons", Waterman, Wiersma, and Bush (1964) concluded that OFF fibers in themselves displayed only initial transient discharges to decreasing illumination. Dimming fibers also respond to frequent or rapid movement or shadows across the receptive field without noticeable adaptation (Wiersma and Yamaguchi 1967a). This property is also shared by sustaining fibers. Their joint response to shadowing is unique, as all other visual fiber classes invariably habituate to such movement (Wiersma and Yanigasawa, 1971). Like dimming fibers, sustaining fibers respond to jittery movement, and both share similar receptive fields (Waterman and Wiersma, 1963; Wiersma and Yamaguchi, 1967a; York and Wiersma, 1975).

Unlike sustaining fibers, however, the receptive field size of dimming fibers in crayfish are smaller in size (Wiersma and Yamaguchi, 1967a). These receptive fields are located predominantly along the central axis of the eyestalk, as either whole eye (total surface), or as a smaller, more localized central receptive fields (Wiersma and Yamaguchi, 1966). Cray-

fish dimming fibers have also been documented along the upper back half of the eye, and also along its top dorsal rim. The discharge rate for a dimming fiber is dependent on the level of background ambient illumination, the absolute light level, and on the preceding relative light level. Yamaguchi and Ohtsuka (1973), in addition to these conclusions, also report that the rate of discharge can be enhanced by illumination of a corneal region complementary to the receptive field. That is, stimulating the appropriate region outside a given receptive field can increase the reactivity of that particular receptive field, by coactivating the surround field for a given fiber type (Wiersma and Yamaguchi, 1967a; Waldrop and Glantz, 1985). Both sustaining fibers and dimming fibers respond to changes in illumination. While sustaining fiber responses have been well documented, dimming fibers have been poorly described. Little is known, for example, of the response characteristics of these cells, nor of associated responses to either purely visual, or to multimodal stimuli. Further, while dimming cell responses appear to be a small subset of all visual responses, their overall significance to the animal requires further investigation. Their infrequent appearance, and possible misidentification may have contributed to the lack of descriptive information regarding these fibers.

The purpose of this research is to study these dimming fibers, and to establish a descriptive catalog of their properties, as determined by extracellular recordings. Wherever these fibers in the optic nerve tract present clearly distinguishable responses, these responses will be described in detail.

MATERIALS and METHODS

Both male and female specimens (carapace breadth 4.5 - 6 cm.) of the low to mid-intertidal Purple Shore crab *Hemigrapsus nudus* were used. The animals were personally collected near San Simeon on the central California coast, and were maintained for research in a recirculating salt-water tank (15° C) under a 12:12 light/dark regimen.

The experimental protocol followed that of previous researchers, and has been adapted to meet current requirements (Waterman and Wiersma, 1963; Waterman,Wiersma and Bush, 1964). Only minor adjustments were made to existing procedures. During experimentation, each subject was immobilized by rubber-banding the legs and cheliped on each side of the body. This prevented the crab from dislodging the recording electrode, and also served to reduce background noise levels from active motor neurons. Also, at the medial and lateral borders of the eyestalk orbit, the carapace was trimmed with clippers. This procedure permitted clearer determination of receptive fields whenever the eyestalk was protectively withdrawn inside the orbit. The immobilized crab was held suspended in air (28° C) by a clamp. A narrow strip of moist toweling covered the mouthparts, permitting gas exchange during the experiment.

Following the protocol of Waterman and Wiersma (1963), the recording electrode was electrolytically etched from a stainless steel insect pin (Elephant brand, #000) to produce a secondary taper to the pin shaft. This taper reduced the overall tip diameter, and helped prevent the electrode from shifting within the optic tract, since the penetrated membranes sealed around the electrode shaft. Usable tip diameters ranged from 1 to 5

microns, and averaged between 3 and 4 microns. A fine copper wire (Beldenamel AWG #38, Belden Cable Co.) was soldered to the pin shaft approximately 1 cm. from the tip. The needle was then coated with a high dielectric compound (Insl-X) to insure overall electrical isolation, in a modification of existing protocol. The coated needle was then baked in an oven (70° C) for a minimum of one hour to assure complete curing of the dielectric compound. The electrode was cut from the needle shaft above the solder joint, and completed by covering the severed end of the shaft and exposed solder joint with acrylic nail polish.

After clamping the prepared animal in the holder, either an #0 or #00 unaltered insect pin was inserted through the fibrous membrane immediately below the medial margin of the proximal eyestalk carapace, at a diagonal along the midline axis towards the ommatidial surface. This additional step not only permitted a better orientation to the optic fibers than did perpendicular penetration, but also helped to protect the recording electrode from incidental damage. The unaltered pin was inserted 2 to 3 mm. within the optic tract, at a level below that of the optic ganglia. No penetrations or recordings were attempted within these ganglia. The probe was removed, and then replaced by the recording electrode. A simple needle ground electrode was passed through the membrane between the metus and carpus joints of the fifth leg. The recording and ground electrode leads passed through a preamplifier (Grass P5) to an oscilloscope (Textronix D10) and audio generator (Grass AM6). The largest cell responses were recorded on a reel-to-reel FM recorder (Ampex SP300), along with a reference time base signal of 10 milliseconds (Textronix 180A)

recorded simultaneously on a separate channel. These taped records were analyzed later on a separate storage oscilloscope (Textronix 564B).

Various response characteristics were cataloged once a fiber type was found and identified. These cells were compared against previous descriptions of visual response modalities (Wiersma, 1966; Wiersma and Yamaguchi, 1966, 1967a; York and Wiersma, 1975). Determination of receptive field size involved correlating evoked action potential discharges to motion passing over the surface of the eye. This was initially accomplished by simple hand or finger-waving over the eye, as performed by Wiersma and Yamaguchi (1966, 1967a). Further determination of receptive field dimensions relied on the use of optical wands that subtended 1° and 5° of arc, respectively, and on a light pen that provided a point source of light (approx. 1°).

Relative illuminance levels were measured by a light meter (Minolta AutoMeter II) and recorded against the Exposure Value (EV) scale. This photographic scale corresponded to the logarithmic decay of light intensities (lux) according to the relationship:

$$\textbf{Illuminance (lux)} = 2.5 \times 2^{EV}$$

Use of the EV scale allowed for greater clarity and simplicity in discussing the relationship of dimming fiber responses to decreasing values of illumination. The relationship between Lux and Exposure Values is presented in Figure 2.

Mechanoreceptive sensitivity was evaluated by stroking the legs and carapace with a camel hair brush. Since the optic ganglia can function as

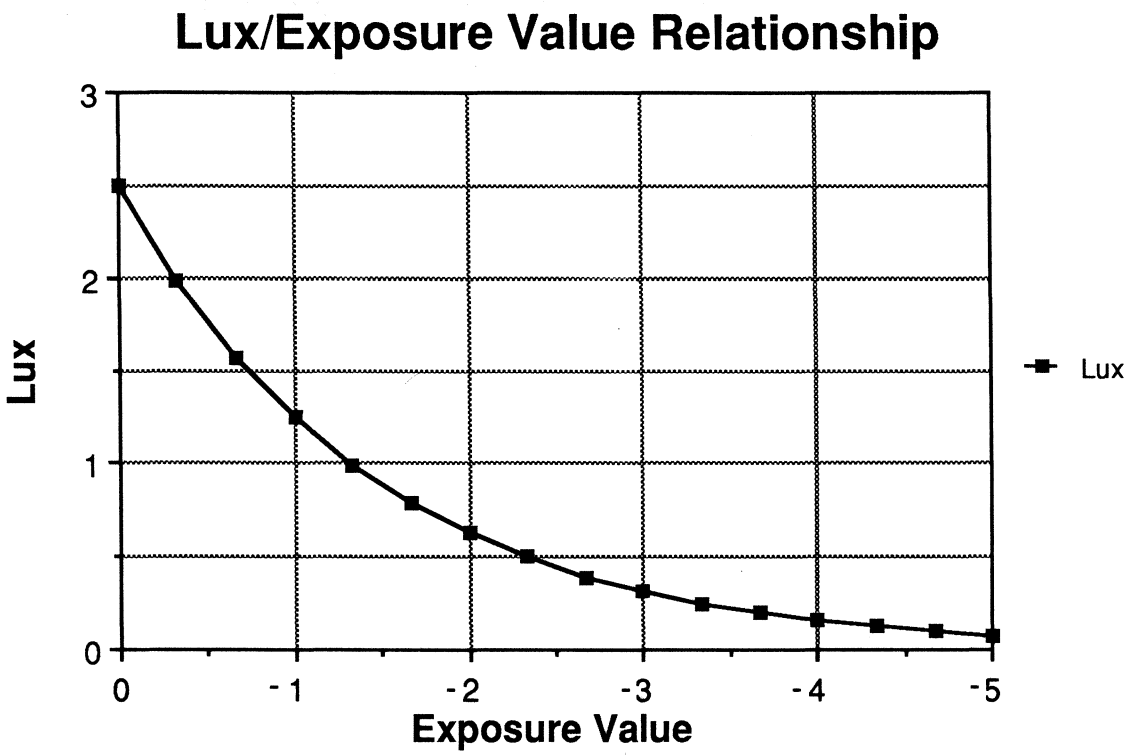


Figure 2. Correspondence between Lux and Exposure Values according to the relationship: $\text{Lux} = 2.5 \times 2^{EV}$

"lateral brains", testing for tactile sensitivity measured any centrifugal optomotor or sensory activity present in the visual pathway (Wiersma, C.A.G., Yanigasawa, K., 1971). Use of the brush prevented any ground path interference.

Once established, a particular cell could remain active for a period of several hours, if the toweling over the mouthparts was moistened periodically with oxygenated saltwater. Although the exact times of duration of each preparation were not recorded, a typical preparation lasted between 3 to 4 hours; the longest for almost 6 hours. It appeared that the duration of a preparation correlated to the overall health of the specimen. Generally, freshly captured crabs lasted longer than those crabs maintained in the research tank for 4 to 6 weeks. Furthermore, the quality of the data obtained late in any preparation diminished. To avoid loss of information, the response parameters were collected within the first half-hour. The preparation often was maintained for several hours to determine if the response characteristics changed with time.

Responses did remain stable until late in the preparation, when the onset of tissue hypoxia led to an increase in the overall background discharge levels. The animal's general responses at this point became weak, accompanied by the diminution and loss of previously observed visual responses. Waterman, Wiersma, and Bush (1964) noted similar concomitant response failures in both the eye and the CNS proper of the crab *Podophthalamus vigil*, as either heart beat or respiration was stopped by excess substitution of the hemolymph by perfusion fluids.

To facilitate experimenter orientation, only the right eyestalk of each crab was used; signals collected from the left eyestalk indicated no differences in observed responses in all visual fiber types encountered. For all cell records except for the first three, only one cell of a single subject was analyzed. Use of only one cell record from a crab prevented repeating a previously examined optic fiber response. All crabs were sacrificed following experimentation.

RESULTS

Analysis of *Hemigrapsus nudus* visual neurons confirmed the presence of dimming cell. These dimming cells are identified based on their capacity to increase their tonic discharge rate relative to decreasing light levels. Information on thirty dimming cells was collected. Parameters for each dimming cell included the visual receptive field, type of movement response, ipsilateral or contralateral hair cell sensitivity, and any distinct repeatable responses over a range of different light intensities.

The observed responses for all visual cell types, including dimming cells, encountered matched descriptions reported by Waterman and Wiersma (1954), Wiersma (1966), and Wiersma and Yamaguchi (1966, 1967a). Non-dimming cell responses were identified throughout the study, but are not discussed here. No differences were observed between records taken from male and female specimens. Signals obtained from the left eyestalk were not different from those of the right eyestalk. Although more than thirty dimming cells were encountered, only the most distinct records are considered in this study.

Receptive Fields

The receptive field for each dimming cell is numbered chronologically in Table 1. The most common receptive field is that of the whole eye receptive field (n=20), which defines the entire ommatidial surface as the area responsive to dimming. These cells correspond to the O50 receptive field described by Wiersma and Yamaguchi (1966). The next most common field

Dimming Cell Characteristics

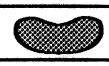
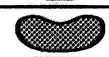
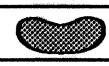
Receptive Fields		Movement Detection	Sensory Sensitivity	Light Response	Enhanced Ranges	Pacemaker Presence
RECEPTIVE FIELD SHAPE		√ : Jittery + : Strong Z plane	+:Ipsi > Contrl 0:Ipsi = Contrl - :Ipsi < Contrl	+: Off > On 0: Off = On - : Off < On OFF: Off Only	BR: Brief Range ER: Extended Range	√: Present
1.		√	+	+	BR	
2.		√	+	+	BR	
3.		X-Y Fast Movement	+	+		
4.	Not Determined	√	+	OFF	BR	
5.		√	+	+	BR	
6.		√	+	+	BR	
7.		√	++	+	BR	
8.		√	+	+		
9.		√	+	+	BR	
10.		√	+	+	ER	
11.		√	+	+	ER	
12.		√	0	OFF		
13.		√	+	OFF		
14.		√	0	OFF	ER	
15.		√	+	+	ER	√

Table 1. Response characteristics of Dimming Cells. The parameters measured receptive field size; movement detection; ipsilateral and contralateral mechano- receptive sensitivity; response to abrupt light changes; enhanced range regions; and pacemaker activity. Dimming Cell Nos. 1, 3, 5, and 8 are variable receptive fields; Nos. 2, 9, 18, 24, and 25 are "Octopus fiber" fields; the remainder represent whole eye receptive fields. Cell No. 4 was not determined (see text).

Dimming Cell Characteristics

Receptive Fields		Movement Detection	Sensory Sensitivity	Light Response	Enhanced Ranges	Pacemaker Presence
RECEPTIVE FIELD SHAPE		√ : Jittery + : Strong Z plane	+:Ipsi > Contrl 0:Ipsi = Contrl -:Ipsi < Contrl	+: Off > On 0: Off = On - : Off < On OFF: Off Only	BR: Brief Range ER: Extended Range	√: Activity Present
16.		√	++	0		
17.		√	+	+		
18.		√	+	OFF	BR	
19.		√	+	0	ER	
20.		√	+	+	ER	√
21.		√	+	OFF	ER	√
22.		√	++	+		√
23.		√	not determined	OFF	ER	√
24.		√	+	OFF		
25.		√	++	OFF	BR	
26.		√	+	OFF	ER	
27.		√	++	OFF	ER	
28.		√	+	+	ER	
29.		√	++	+	ER	
30.		√	++	OFF	BR	√

Table 1 (continued). Response characteristics of Dimming Cells. The parameters measured receptive field size; movement detection; ipsilateral and contralateral mechanoreceptive sensitivity; response to abrupt light changes; enhanced range regions; and pacemaker activity. Dimming Cell Nos. 1, 3, 5, and 8 are variable receptive fields; Nos. 2, 9, 18, 24, and 25 are "Octopus fiber" fields; the remainder represent whole eye receptive fields. Cell No. 4 was not determined (see text).

present was the octopus fiber (n=5). The response characteristics for this fiber type were first described by Wiersma (1966). The octopus receptive field is not constant or regular; instead, it oscillates over time. The limits of this particular field shifts periodically between two extremes. At its smallest defined field, the octopus fiber maps a circular field, whose radius is from the apparent center of the eye, covering between one-half to one-third of the total ommatidial surface. At its maximum, the field radius is larger, covering roughly two-thirds of the whole eye surface. The exact borders of this field remains undefined; as the fiber response changes, the receptive field described by the response changes concomitantly.

The remaining five receptive fields were not repeated during the experiments. Two of these cells appeared to match dimming cells previously described by Wiersma and Yamaguchi (1966). Cell No.1 corresponded to the description of O86, and cell No.8 matched that of O83. Cell No.4 had no receptive field identified, since it appeared to respond to both whole eye and octopus fiber receptive fields. Because of its otherwise clear response to dimming stimuli, this cell is included in the study although its receptive field remains undetermined.

Movement Responses

Responses to movement within the receptive field of each cell indicated that most cells (n=29) shared a common "jittery" response. Jittery movement responses, as described by Wiersma (1966), indicates an ability to discriminate both rapid and slow target movement on all three spatial planes with no directional preference. When an object remained stable within the receptive field, the cell's firing rate returned to its previous level,

as the cell adapted to the stationary target. Subsequent target movement caused the cell to respond again with increased discharges to the apparent shift, or "jitter" in the target's spatial position. The single exception was cell No.3 which responded only to fast movement in the X-Y plane.

Mechanoreception

The majority of cells (n=26) indicated a greater mechanoreceptive ipsilateral sensitivity than for contralateral sensitivity. Brushing the hair cells of the carapace, cheliped, or legs increased the action potential frequency in the active "dimming" cell. Testing was done both before and during the course of the experiment.

Responses to Light Termination or Onset

Dimming cells also responded to abrupt changes in light intensity, both to its onset and offset. Most cells (n=28), responded more vigorously to light termination (offset), while only two cells demonstrated equal responses to both light onset and offset. No cell in the study was observed to have a greater response to light onset versus light offset.

Pacemaker Cell Activity

"Pacemaker" responses were also noted among dimming cells (n=6). With the lights off for a period of time, rhythmic pulsatile discharges became distinct. These pacemaker cells started firing after a brief lag of thirty seconds to a minute following the onset of darkness. Brushing carapace hair cells, or presenting a light flash inhibited pacemaker activity for one to two minutes; the pacemaker response then recovered.

Uninterrupted pacemaker discharges continued for a variable amount of time, ranging from one to five minutes in duration. Past this point, the response became indistinct against the normal background activity.

Dimming cell pacemaker activity was noted only occasionally, and described only when clearly identifiable. No correlation between the visual stimuli presented in the experiments and pacemaker activity was established.

Dimming Cell Population Characteristics

Except for pacemaker responses, which had variable and ill-defined activity, all other dimming cell responses in the study population remained stable and consistent during each experiment. Visual responses became attenuated and inconsistent only at the later stages of an experiment, when the animal's general health and nervous activity declined as previously described.

Data from this study is plotted as number of active cells present at a given Exposure Value (EV). For the dimming cell population, Figure 3 indicates that a greater number of cells are found at -5EV, the lower limit of measurable sensitivity of the light meter used. Only 29 of the total thirty cells in the study are shown in Figure 2, since cell No.9 began its observable response at -5EV, and continued to lower immeasurable levels approaching total darkness. Because of this extreme range, cell No.9 is present in Table 1, but is not included in Figure 3.

In considering the total cell population, a directly proportional relationship exists between the number of active dimming cells and increasing darkness. Due to the low sample size, no statistical tests were

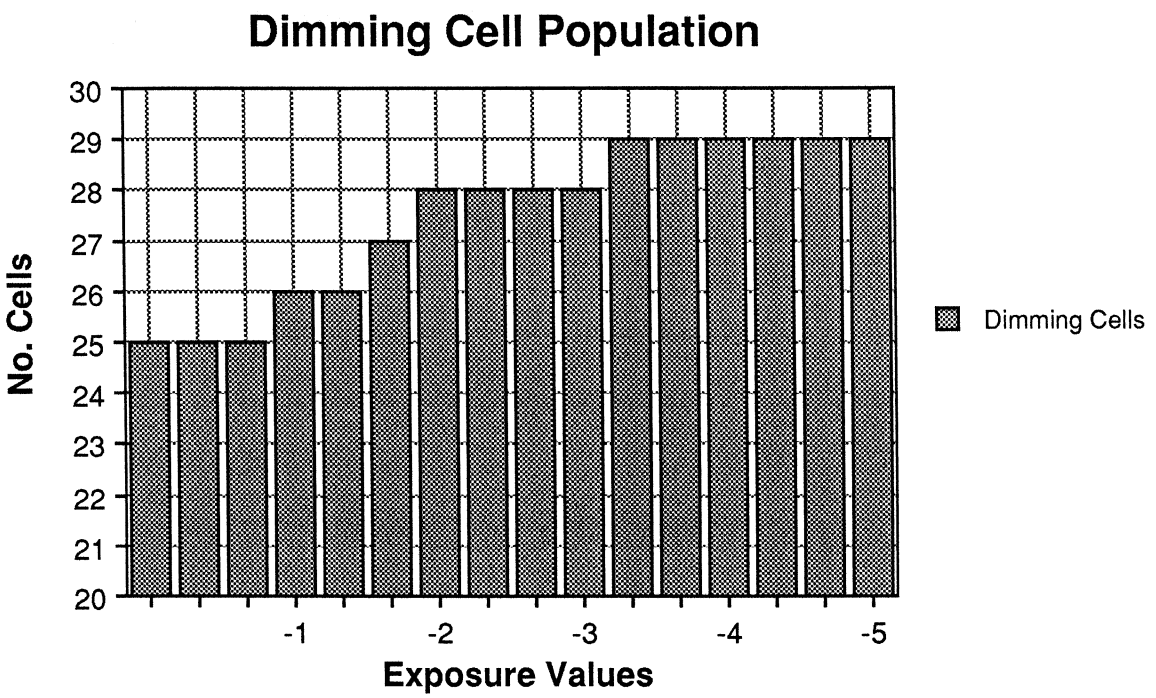


Figure 3. Dimming Cell population response to different light levels. Each vertical bar represents a discreet measurement of the total number of Dimming Cells present at that specific light level.

performed on the data. Despite this lack of statistical analysis, Figure 3 strongly suggests that an increasing number of dimming cells are present with increasing darkness.

Brief Range and Extended Range Responses

A contribution of this research has been the identification of enhanced ranges of sensitivity in dimming cells. While all cells of the population responded over a range of light levels, a subset of these dimming cells increased their discharge frequency abruptly after passing some specific light level (Figure 4). This increase in discharge rate was related to distinct thresholds of illuminance. Further, these threshold values were consistent in each cell, and remained independent of the rate of dimming both above and below the threshold level.

In the study population, the majority (n=23) exhibited this enhanced range sensitivity. This group of dimming cells can be further classified, based on the relative extent or range of enhanced sensitivity. These response subtypes of dimming cells will be considered as Brief Range (BR), and Extended Range (ER) responses.

Dimming cells with Brief Range (BR) responses (n=11) were found to have increased discharge rates only at specific light values. These threshold levels are quite distinct, and clearly represented specific illuminance values. For each cell, the discharge frequency increased only at the observed level, and returned to the normal relative level of activity on either side of the threshold value. BR responses sometimes occurred more than once in a particular cell (n=3). All four of these cells were consistent in having a second BR response at -5EV. Extended Range (ER) responding

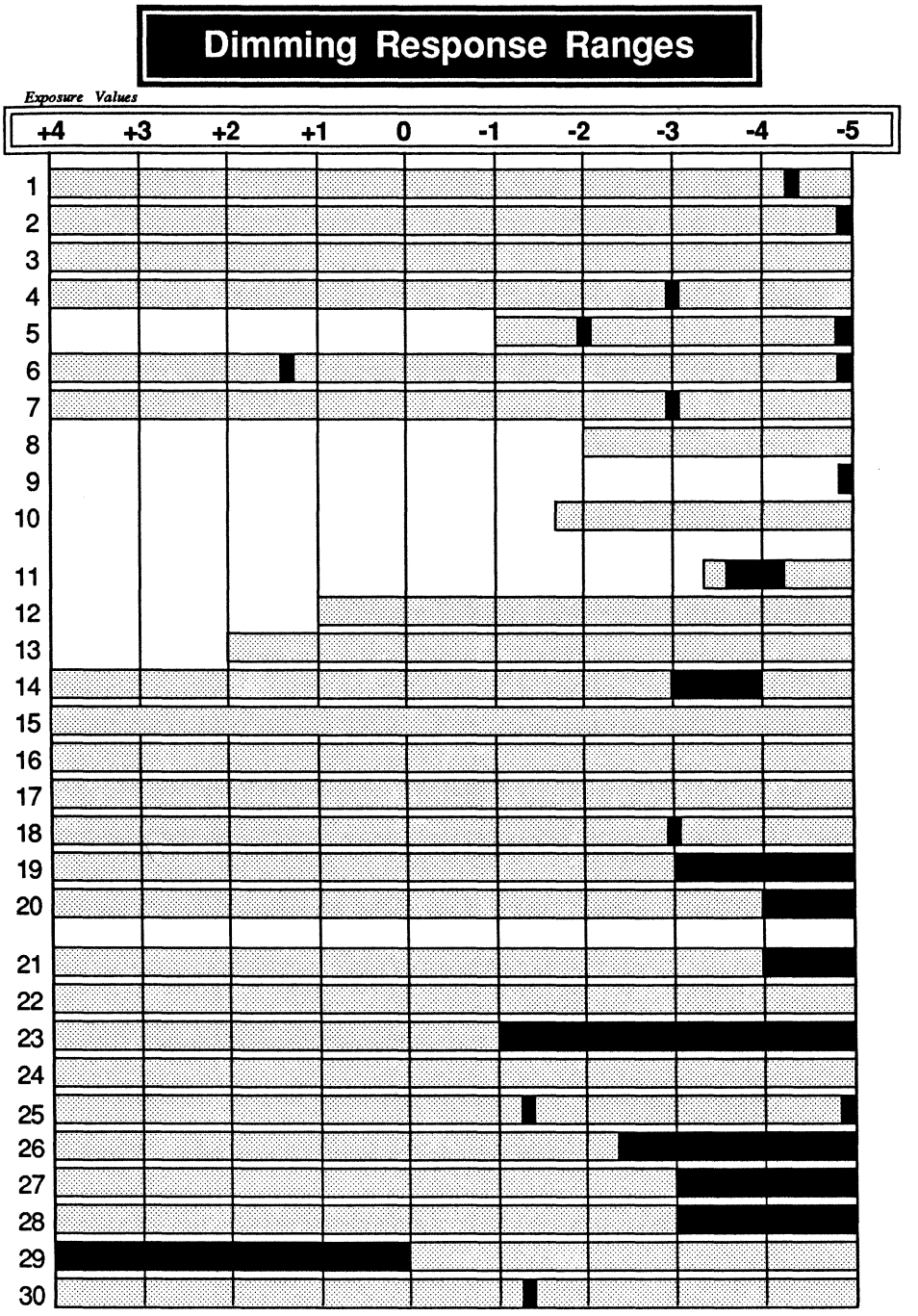


Figure 4. Distribution of Dimming Cell responses over the measured range of light values (EV). Shaded horizontal bars represent the limits of the observed visual response; the black banding within indicate areas of increased discharge sensitivity to certain EV levels. The narrow black bands are Brief Range (BR) responses; the wider black bands are Extended Range (ER) responses.

cells (n=12) maintained their increased discharge rates over a well-defined range of light levels. This characteristic served to separate the ER response from the BR response, which had distinct and isolated values of increased sensitivity. The majority of ER cells (n=10) continued their enhanced discharge rates from a specific light level through to -5EV, the lowest light level measured. The other two ER responses in the population were found to end their ranges at some value above -5EV, returning to their non-enhanced discharge rate beyond that value. The distribution of both BR and ER responding cells relative to the total dimming cell population is presented in Figure 5. Both types of Range responses are present at lower illumination levels, with the exception of cell No.29 (Figure 4), which had an ER response between +4 to 0EV. Figure 6 represents the number of each response subtype present at different light levels. BR responses appear more variable in the dimming cell population, while ER responding cells consistently are more apparent at lower light levels.

Off-only and Off/On Light Responses

Another contribution of this research has been the study of associated responses to both light onset and offset in dimming cells. Dimming cell responses to both 1)the termination of light; and 2)the onset of light after a period of darkness, indicated differences within the total population.

All dimming cells in this study (n=30) responded to abrupt changes in light levels. The most common response (n=16) was to both the offset and onset of light. These cells reacted to abrupt light level changes with a brief increase in discharge frequency that adapted rapidly. Because they preferentially had stronger responses to light offset than to onset, they will be

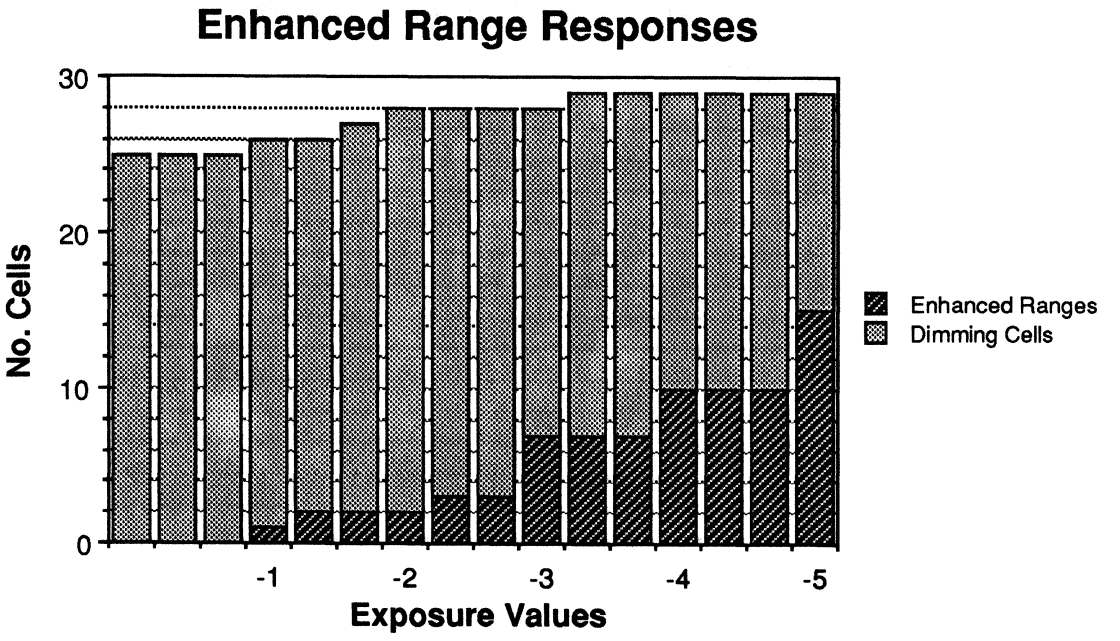


Figure 5. Distribution of Enhanced Sensitivity Cells relative to the total Dimming Cell population. These Enhanced Sensitivity Cells are comprised of Brief and Extended Range Cells.

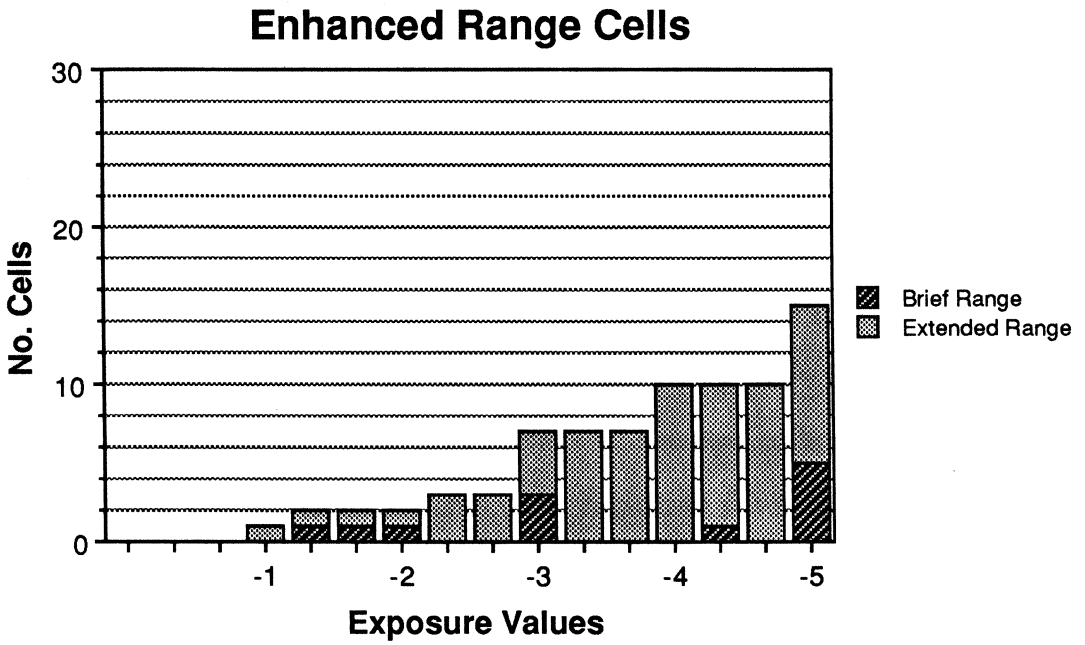


Figure 6. Distribution of Brief Range (BR) and Extended Range (ER) responding cells. The total number of cells with Enhanced Range sensitivity is given by the total height of the bar; the relative contribution of BR and ER cells is represented in shading (see figure legend).

considered as "Off/On" responses. Figure 7 suggests that, for this response, more cells are active at lower light levels.

The other subset of dimming cells reacted to light offset only (n=12). Turning the lights back on abruptly or gradually had no effect on the level of activity. These "OFF-only" responses were found to maintain a constant population throughout all light levels (Figure 8). In testing for light offset or onset responses in BR or ER identified dimming cells, 9 of the 12 OFF-only cells were found to have enhanced sensitivity for changes in relative illumination. In Figure 9, no distinction is made between BR or ER responses, but instead are considered together. Again there is a consistent increase in the number of active cells and increasing darkness.

The remaining two dimming cells in the study were found to be equally responsive to light onset and light offset. These two cells are therefore excluded from the previous figures. No variation from the response characteristics for either Off/On or OFF-only were noted in these two cells.

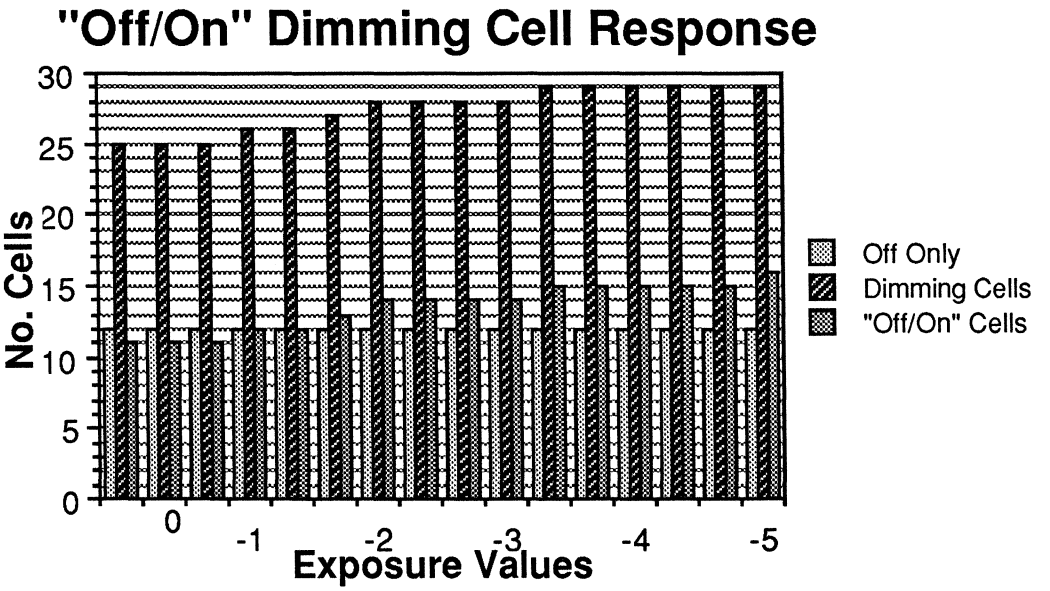


Figure 7. Distribution of "Off/On" responding Dimming Cells relative to the OFF-only cell response, and to the total Dimming Cell population.

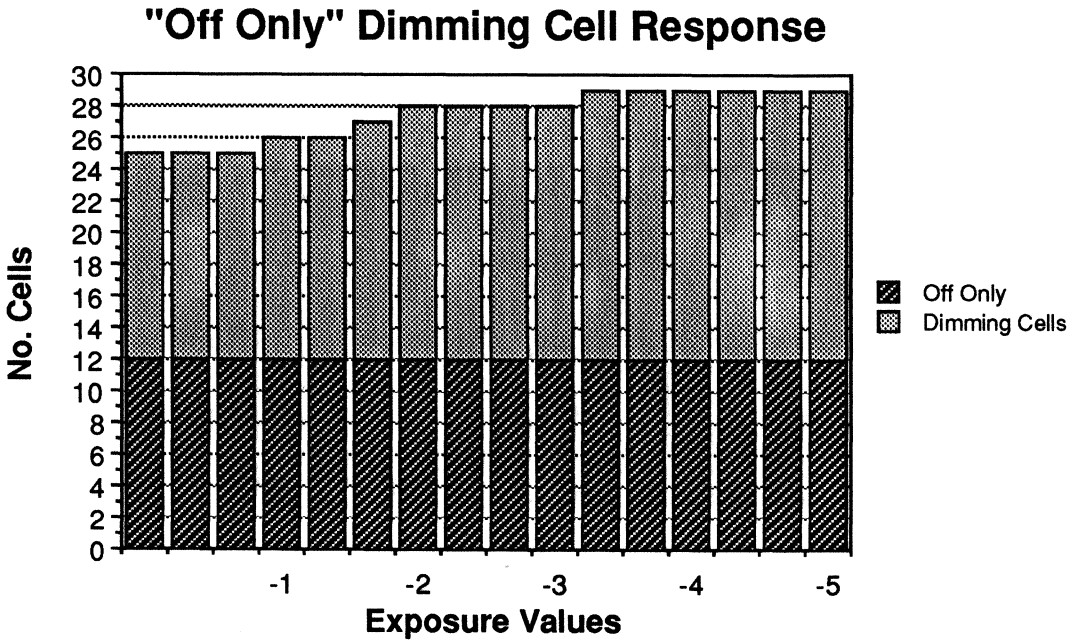


Figure 8. Distribution of Dimming Cells with "OFF only" responses, relative to the total cell population.

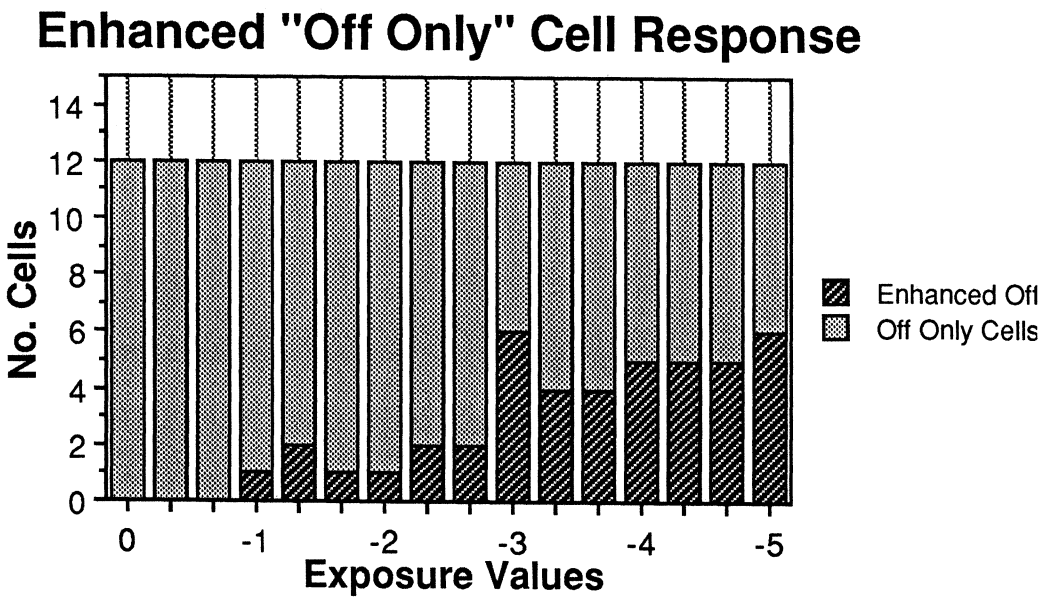


Figure 9. Distribution of OFF only cells with Enhanced Range responses (both BR and ER) relative to the OFF cell population.

DISCUSSION

These results indicate that dimming cells possess distinct and characteristic responses. These cells clearly exist in *Hemigrapsus nudus* and show that at the level of the optic tract, dimming responses have been processed by preceding neurons. The various responses recorded for these cells suggest that different responses are processed simultaneously from a given receptor.

Receptive Fields

Dimming cell characteristics correlated well with previous research, although analysis of their associated receptive fields reveals a notable exception. As discussed before, twenty of the thirty dimming cells in *Hemigrapsus nudus* had whole eye receptive fields, and two other cells were found to match receptive fields described by Wiersma and Yamaguchi (1966). These researchers identified 41 occurrences of homolateral dimming fiber responses. Fully half of these cells (n=21) were identified as the O79 receptive field, covering the upper back quadrant of the eye; only 20% (n=8) identified the crayfish whole eye receptive field O50.

Other studies in crayfish (Yamaguchi and Ohtsuka, 1973; Waldrop and Glantz, 1985) and goldfish (Northmore, Williams and Vanegas, 1983; Northmore, 1984) indicate that dimming cell receptive fields are also found centered in a band along the horizontal midline, or else over the upper dorsal half of the eye. The striking predominance of whole eye receptive fields in *Hemigrapsus* may be explained in part by their habitat. Whereas

crayfish enjoy a wide distribution in more aquatic freshwater niches, *Hemigrapsus nudus* is a prominent indicator species of the rocky mid-to-high intertidal zone of the Pacific coastline (Ricketts, Calvin and Hedgepeth, 1985). These crabs typically live under large rocks, avoiding predators in the open. In such a visually complex environment, dimming cells in *Hemigrapsus* may serve to pool all available light. Since feature perception would be at a minimum under such conditions, the discrimination of object movement by means of shadow or total illuminance changes may be critical for dimming cells.

The present research documents two observations of *Hemigrapsus* receptive fields. The first is the clear majority of whole eye fields; the second is the identification of octopus fiber receptive fields among dimming cell responses. The exact significance or need for an oscillating receptive field remains unclear, although it is possible that this field may extract a higher sensitivity to changes in relative illuminance, or to shadow movement, from its differential field sizes. Further experiments with these cells is necessary to fully comprehend this response.

Mechanoreception

Many cells in the study displayed multimodal properties-- that is, the ability to respond to both visual and non-visual mechanoreceptive (tactile) stimuli. This finding recalls earlier findings by Waterman and Wiersma (1963), and Waterman, Wiersma and Bush (1964). Their research on the Hawaiian swimming crab *Podopthalmus vigil* established visual interneurons having mixed response modalities. One pertinent feature in their study that they observed was mechanoreceptive sensitivity.

How this sensitivity relates to the visual processing of dimming cells is not apparent, although the results in Table 1 show that all dimming cells in the study exhibited clear associations with other responses, both visual and non-visual. This correlation demonstrates that dimming cells are not pure visual fibers, but instead are capable of processing visual and non-visual information concurrently.

Crayfish studies indicate that the optic nerve tract has a distinct organization. In cross-section, different fiber types are separate and localized. Further, approximately two-thirds of all tract fibers are centrifugal, carrying motor optokinetic and sensory mechanoreceptive information from the central supraesophageal ganglion to the eyestalk (Wiersma, 1966; Wiersma and Yamaguchi, 1966). In *Hemigrapsus*, no such organization exists; all fibers types are mixed throughout. Indeed, the presence of mechanoreceptive sensitivity in *Hemigrapsus nudus* is noteworthy, since experimentally, the mechanosensitivity can be confirmed; yet histological preparations indicate a smaller proportion of actual centrifugal fibers than that expected from the observed response (Rafuse; personal comm.).

Enhanced Range Responses

The existence of enhanced ranges of sensitivity in dimming cells has not been reported in the literature. The present study repeatably confirmed their presence, and the observed response can be separated into two distinct classes. The first is the Brief Range (BR) response. This dimming cell response appears in a small proportion of the study population. When encountered, BR responding cells demonstrate a consistent increase in discharge rate, which had no correlation to any experimental stimuli.

BR responding dimming cells typically were noted at narrow ranges of EV light values, while the Extended Range (ER) class of responses increased their tonic discharge rate over a wide range of values (Figure 4). Both ER and BR responses shared similar increases in discharge rates, but they were separated based on the extent or range of the enhanced response. While Figure 5 indicated that both dimming response subtypes are more prevalent at levels of increasing darkness, the enhanced range noted for Cell No.29 in Figure 4 provides a clear exception to this generalization.

It was noted throughout the results that the number of active cell responses for enhanced range and all other cell types-- except OFF-only cells-- appeared to increase proportionately at -3EV (lux=0.31). Whether this observation is significant must be deferred until a larger sample size can be generated for a meaningful statistical interpretation of this perceived trend.

Movement Responses

The prominent dimming cell response to jittery movement noted in the study is well documented in the literature (Wiersma and Mill, 1965; Wiersma and Yamaguchi, 1967a,b; Wiersma and Yanigasawa, 1971; York and Wiersma, 1975). While jittery movement responses can occur in all classes of visually responding cells, this response is considered special in both dimming and sustaining fibers. These two fiber types respond to jittery movement without noticeable adaptation, while all other visual fibers "invariably habituate to such fast shadowing" (Wiersma and Yanigasawa, 1971). Dimming and sustaining fibers may therefore maintain jittery movement responses as a means of determining object location, rather

than for specific feature or detail recognition. In *Hemigrapsus*, the associated jittery movement response of dimming cells matches reports from other crustacea. Like the rock lobster, this jittery response may increase general alertness levels regarding their environment rather than individual component features (York and Wiersma, 1975).

Light Off and On Responses

Another related characteristic of dimming cells was their detection of light onset and offset. Dimming cell responses have generally been considered as distinct from "Off/On" cells in the literature (Waterman, Wiersma and Bush, 1964; Wiersma, 1966; York and Wiersma, 1975). Yet the concomitant presence of responses to abrupt light onset and termination in dimming cells suggest different possibilities. One may be that Waldrop and Glantz's (1985) classification of dimming fibers as a "tonic OFF optic tract interneuron" may be more applicable; however, it cannot satisfactorily explain the observed capacity to measure light onset from darkness. It is clear from Table 1 that different types of light responses were established: response to both Off and On (Off/On); OFF-only responses; and equal responses to both conditions. To use Waldrop and Glantz's definition would exclude 60% (n=18) of the dimming cells in the present study. It would be more reasonable instead to consider the detection of light onset and offset as an additional response parameter of the multimodal dimming cell.

Off and On responses to light have been noted in a number of invertebrate and vertebrate studies, where the vertebrate system response is mediated by retinal ganglion cells for information regarding contrast

sensitivity and object spatial position (Waterman and Wiersma, 1963; Wiersma and Yamaguchi, 1967a; York and Wiersma, 1975; Hammond and Mackay, 1983; Ashmore and Copenhagen, 1983; Sclar, Ohzawa and Freeman, 1985). In vertebrates, the visual response is a product of interacting visual interneurons of a given receptive field (Bailey and Gouras, 1985). Similar interneuron relationships exist in invertebrates that not only may explain pure "On" and "Off" responses to light, but also the activity of dimming and sustaining fibers.

Dimming Fiber and Sustaining Fiber Relationships

Similarities in dimming and sustaining fiber properties have been recorded in previous research. An interesting observation from this literature has been the difficulty of studying dimming responses separate from those of sustaining fiber responses (Wiersma and Mill, 1965; Wiersma, 1966; Wiersma and Yamaguchi, 1967a,b; Wiersma and Yanigasawa, 1971; Yamaguchi and Ohtsuka, 1973; York and Wiersma, 1975). Although dimming and sustaining fibers display opposite responses to light, they remain closely related in position (Wiersma and Yamaguchi, 1967a). In addition, their response characteristics are quite similar.

Dimming cell and sustaining cell responses share traits beyond that of jittery movement responses. Several studies concluded that sustaining fiber receptive fields are larger in size, and comprise larger diameter fibers of greater discharge amplitude than dimming fibers (Wiersma, 1966; Wiersma and Yamaguchi, 1966; Yamaguchi and Ohtsuka, 1973). Considering the close proximity noted for these two fiber types, the diffi-

culties in analyzing dimming cell properties reported by these researchers can be understood.

The present study, however, relates information about receptive field size that differs from past reports in other crustacea. The receptive field most commonly encountered was the whole eye receptive field, which was larger than those of sustaining fibers examined. While precise sustaining fiber records were not kept, all sustaining fibers identified in this study had receptive fields that were consistently smaller than those of dimming cells. Sustaining fiber receptive fields most often observed defined the central two-thirds of the eye (O1), the ventral half (O9), and the medial and lateral dorsal quadrant receptive fields (O21 and O2) identified by Wiersma and Yamaguchi (1966). The appearance of sustaining fibers in the study was highly variable; yet in all cases, receptive field sizes were smaller than those of dimming fibers. Although the dimming cell octopus fiber receptive field covers roughly the same surface area as sustaining field O1, by far the greater proportion of cells in this study had whole eye fields (n=20). Only for five cells in the dimming population can sustaining fiber fields be considered larger.

The discrepancy in the present results and past research is unresolved. Any factor, from an insufficient sample size, to an actual higher proportion of whole eye dimming fields in *Hemigrapsus nudus*, may account for these observations.

Dimming Models in Other Species

That dimming cells do in fact exist in *Hemigrapsus* is apparent from the present results and observations. While well documented among

crustacea, dimming responses have also been reported in a number of other invertebrate and vertebrate species, including the Lubber grasshopper (Northrop and Quinon, 1970), Orb Weaving spiders (Yamashita and Tateda, 1981, 1983), turtles (Ashmore and Copenhagen, 1983; Marchiafava, 1983), goldfish (Northmore,Williams, and Vanegas, 1983; Northmore, 1984), and catfish (Sakuranaga and Naka,1985). Among these and other animal studies, different mechanisms for the activity of dimming responses have been proposed. The mechanisms considered here all attempt to relate the observed physiologic responses to light, to underlying anatomical connections and activity.

In early reports by Waterman et al. (1963, 1964), it was apparent that the visual responses measured from optic tract interneurons represented the transmission of a highly integrated channel of information: for a given visual field, several hundred ommatidia contribute to the primary sensory perception. How this perception of a stimulus is processed in the retina, and transmitted through the optic ganglia remains uncertain. In crustacea, integration may occur through parallel routes, whereas vertebrate processing is generalized to be in series (Waterman,Wiersma and Bush, 1963; Yamaguchi and Ohtsuka, 1973; York and Wiersma, 1975; Waldrop and Glantz, 1985).

Given the close relationships established between sustaining and dimming fibers, any model of dimming cell activity must also consider sustaining fiber responses. Previous studies in barnacle, catfish, and crayfish suggest possible connections between visually active interneurons. These connections will be used to postulate a mechanism for dimming activity in *Hemigrapsus*. As all three of these animals have documented

dimming responses, a brief summary of key features will be presented for each species, and then will be followed by proposition of a dimming mechanism for *Hemigrapsus*.

Barnacle Vision

Barnacle visual properties provides one such model for consideration. Shadows passing over barnacle photoreceptors elicit a withdrawal reflex of the animal into the shell. As studied by Stuart and Oertel (1978), this response comprises a three neuron serial pathway. The median eye detects changes in light intensity; this information is spread decrementally to the second order I cell; and then to the third order A cell before being conducted to the ventral nerve ganglion that mediates the muscle neurons associated with shell closure. In this pathway, the reflex is mediated by the activity of the second order I cell, which is held hyperpolarized in light, and is depolarized with the decrease or termination of light detected by the primary photoreceptor. The A cell reacts similarly to information conveyed to it by the I cell, but is more strongly depolarized in response to light diminution. Thus the I (inverting) cell transmits light dimming information to the A (amplifying) cell.

Expanding on their previous findings, Oertel and Stuart (1981) found that dimming of light did in fact depolarize the I and A cells serially. They concluded that this activity was controlled through voltage-gated Ca^{2+} channels in the I cell membrane in response to the offset of light hyperpolarization; that is, the release from light inhibition.

Catfish Amacrine Cells

A similar vertebrate response was studied in catfish "amacrine" (axonless) cells (Sakuranaga and Naka, 1985). These researchers studied responses from the sustained class of amacrine type-N cells that were stimulated by light dimming. As in the postulated mechanism for barnacles, these cells responded with sustained hyperpolarization of resting membrane potentials to light bursts, and with pronounced depolarization to dimming. Type-N cells typically inverted the visual signal received from horizontal and bipolar cells, depolarizing only with decreasing in overall illumination. The authors suggested that this line of transmission allows the detection of "complex temporal patterns" in the visual input.

Crayfish Surround Inhibition

Another set of local interneurons in crayfish has been studied. A review of this particular mechanism suggests another possible model for dimming responses in crustacea. In examining the surround inhibition properties of crayfish sustaining fibers, Waldrop and Glantz (1985) describe the influence of local interneurons defined as "amacrine" cells. This term is somewhat misleading, as amacrine cells are more properly discussed in vertebrate visual organization. These crayfish interneurons are so named, however, for their lack of a projecting axonal process. Regardless of this terminology, "amacrine" cells are local interneurons with extensive horizontal bidendritic processes in the external medullary layer. These cells are also characterized by sustained hyperpolarization to light, and depolarization to its diminution. These "amacrine" cells acted as an

inhibitory surround for sustaining fibers, where illumination of a complementary region of these "amacrine" cells reduced the frequency of concomitant sustaining fiber recordings. These cells also affected dimming cells conversely, by increasing dimming discharges from illumination of the "amacrine" surround. In its response, "amacrine" cells behave functionally like the barnacle I cells and the type-N cells of the catfish by inverting the primary response of light.

Crayfish Dimming and Sustaining Fibers

A comprehensive dimming mechanism is proposed by Yamaguchi and Ohtsuka (1973). The various component features presented in the above mechanisms is capably integrated in this mechanism. These researchers attempt to relate both the neural connections and physiological responses of both dimming fibers and sustaining fibers. Yamaguchi and Ohtsuka propose that visual information from both fiber classes are processed independently via parallel channels, and are linked by reciprocal inhibitory pathways modulated by light intensity. This organization serves to explain the various functional similarities in responses between the two fiber types, and also their apparent reciprocal light responses. In their model, the surround inhibition is modulated by the other fiber type-- here, local interneurons such as Waldrop and Glantz's "amacrine" cell could mediate the observed coactivation of the reciprocal system by inverting the primary photoreceptor response through hyperpolarization. In analyzing factors affecting the discharge frequency of both fiber types, the researchers determined that the relative light level, both present and preceding, the ambient background light level, and reciprocal coactivation between fiber types were

critical in determining the discharge rates for dimming and sustaining fibers.

Dimming Model for *Hemigrapsus*

Yamaguchi and Ohtsuka's (1973) proposed mechanism offers much towards developing a dimming cell model in *Hemigrapsus*. It is noteworthy that their mechanism focuses on the dimming and sustaining fiber interactions observed in the crayfish, since more recent work suggests that this interaction indeed is firmly established between the two cell types. *Hemigrapsus nudus* studies by Huang (1981) and Rafuse (personal comm.) indicate that a dimming cell bipolar dendritic interneuron is located in the medulla externalis--the same ganglia layer as the "amacrine" interneuron observed by Waldrop and Glantz (1985) in sustaining fibers. Although the two fiber types share a common interneuron location, the interneurons are not identical. The "amacrine" interneuron has extensive dendritic branching in the most distal region of the medulla externalis; in dimming cells, the bipolar interneuron has extensive branching in two distinct levels in the medulla externalis, with both levels more proximal in position than that of the sustaining fiber "amacrine" cell. Here it is important to consider that the synaptic integrity of each cell response remains whole, although collateral connections may occur between the two fiber types.

Based on these findings, it may be proposed that the neuronal circuitry of both dimming and sustaining fiber responses are mediated in similar ways, but through separate pathways. Dimming fiber responses may then be mediated by a second-order interneuron with bi-level branching. Inhibition then may indeed be the key to understanding dimming activity,

since inhibitory pathways typically require an additional synaptic connection, relative to excitatory pathways. Dimming cell responses would then represent a three or four neuron path from the retinal cell, to the dendritic layer(s) of the bipolar interneuron, and then to the optic tract interneuron. Sustaining cell responses, however, would also present a three neuron path: retinal cell; "amacrine" cell; optic tract interneuron. If coactivation, similar to that discussed by Yamaguchi and Ohtsuka (1973), was present, then any collateral interconnections between the two groups can account for the surround inhibition described by Waldrop and Glantz (1985) in sustaining fibers, and also the coactivation of receptive fields that overlap or remain outside a given dimming receptive field described by York and Wiersma (1975) and Yamaguchi and Ohtsuka (1973).

Visual dimming responses in *Hemigrapsus* can then be considered as a serially conducted pathway with associated connections with a separate parallel pathway for sustaining fiber responses.

Dimming Cells: Visual Significance

For *Hemigrapsus*, its rocky mid-intertidal environment is visually complex. The ability to discriminate changes in light intensity may serve well to detect the displacement of an object within its visual field. If this is true, then the multimodal properties of dimming cells could be explained. If dimming cells detect illuminance changes caused by object shadow or outline movement, then the observed coupling between jittery movement perception and dimming fiber activity may increase awareness of object movements within their surroundings.

Recent research into the visual environment of semiterrestrial Brachyuran crabs suggest that different mechanisms are responsible for vision between the two main groups: broad-fronted species having short eyestalks, widely separated; and narrow-fronted species with close set, vertically elongated eyestalks (Zeil,Nalbach and Nalbach, 1986). The authors consider that in the relatively flat and open habitats of narrow-fronted species, such as the Ocypodid Fiddler crab, an acute vertical resolving power determines object size and position. For broad-fronted Grapsid species, like *Hemigrapsus*, no such specialization exists. Instead, to analyze their more optically complex rocky intertidal habitat, other dimensional cues and mechanisms, including binocular vision (stereopsis), appear more important.

Although Zeil, Nalbach and Nalbach (1986) strongly implicate the role of stereopsis for grapsid vision, the conclusion remains tenuous. Yet one of the other possible mechanisms may involve dimming cell responses. The ability to discriminate both shadows through illuminance changes, and movement via jittery responses, would be vital in comprehending object displacement in low light environments.

Additionally, feature perception at low light levels is reduced as less visual information is available. Nalbach, Thier and Varju (1985) document that the pooling of low light visual information gains in importance for mediating optokinetic (eyestalk movement) sensitivity. Enhancement of orientation and tracking systems through dimming cell activity would be advantageous under these conditions. Dimming fibers appear then to be involved in mediating visual information at levels of low illuminance. The multimodal responses observed in dimming cells may therefore be the

result of coupling of simultaneous stimuli under low light conditions. These multimodal responses include enhanced light and mechanoreceptive sensitivity, and responses to light onset and termination. Further, while an exact mechanism for activity remains uncertain, recurrent inhibition is a strong possibility. The presence of horizontal cells in the medulla externalis may be responsible for processing dimming information before transmission to interneurons in the optic nerve tract.

SUMMARY

Extracellular recordings from optic tract interneurons of *Hemigrapsus nudus* prove the existence of dimming responses. These dimming cells respond to decremental decreases in ambient light levels, and appear to have primarily whole eye receptive fields. Associated with these fibers are different simultaneous responses, involving perception of jittery movement, mechanoreceptive sensitivity, and light on/off detection. Dimming cell populations are present in greater numbers at decreasing light levels, and have consistent, repeatable responses. Although no mechanism for activity is established, a pathway involving recurrent inhibition may be responsible for the processing of dimming responses. This pathway may involve the horizontal cells of the medulla externalis as the second order interneuron between photoreceptors and tertiary interneurons higher in the optic nerve tract.

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