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Inhibitory Fields in the Limulus Lateral Eye

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INHIBITORY FIELDS IN THE *LIMULUS* LATERAL EYE

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by

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Preface

I would like to express my gratitute to the administration of Rockefeller University and especially to Dr. Detlev Bronk for the opportunity to participate in the graduate program and for their generous support of my family and myself. Also, I would like to thank the students and faculty of the University who together provided a stimulating atmosphere for the pursuit of a graduate education.

My deepest personal thanks go to my advisors, Drs. H. Keffer Hartline and Floyd Ratliff. The rich experience of working with them in the laboratory and of discussing with them the many aspects of this work have been invaluable to me.

I am indebted to Dr. Don Quarles, research mathematician at the IBM Watson Research Center, Yorktown Heights, New York for the theoretical analysis contained in this thesis. Some of his many contributions are cited within the text. I thank him for his generous assistance and uncountable hours of helpful discussions.

There are many others to whom thanks are due: Drs. David Lange, Fred Dodge, and Bruce Knight for their very helpful suggestions which are too numerous to list; to Dr. Victor Wilson and the thesis committee for their instructive comments; to the skilled instrument makers, Werner Krug and Hans Braun; to the American Optical Company for supplying the fiber optic instruments; to my wife for typing the early drafts of the thesis and Mrs. Richard Costello for typing the final draft; and to Miss Ruth Mandlebaum and the illustration service for their artistry in preparing the figures.

For the patience and understanding of my wife and family I am forever grateful.

Summary

The spatial distribution of the inhibitory influences exerted by ommatidia in the Limulus lateral eye was measured. The source of inhibition was a small cluster of ommatidia illuminated through a flexible bundle of glass fibers ("fiber optics"). The inhibitory field of the cluster was determined by measuring the decrease it produced in the response frequency of surrounding ommatidia which were illuminated individually through single glass fibers. Applied directly to the corneal facets of the ommatidia, the single fibers provided unusually effective stimulation with a minimum of light scatter into adjacent receptors.

The inhibitory field is elliptically shaped with its major axis in the antero-posterior direction on the eye. In the adult animal the field covers an area of 15 mm^2 (about 30% of the eye) and contains approximately 300 ommatidia; however, less than one-third of that number receives the bulk (75%) of the inhibitory effects exerted by the small cluster in the center. The position of maximum inhibition is located at some distance from the center of the field: 0.8 mm or 3 ommatidial diameters in the dorso-ventral direction and 1.3 mm or 5 ommatidial diameters in the antero-posterior. The inhibitory effect tapers off toward the periphery becoming negligible at approximately 2 mm from the center of the field in the dorso-ventral direction and at 3.3 mm in the ventro-posterior direction. The configuration of the field was found to be similar for a number of experiments in which the source of inhibition was located in various positions on the eye.

Control experiments show that the diminution of the inhibitory effect near the center of the field is not an artifact of the measuring technique and cannot be readily explained by local competing excitatory processes.

The ommatidial inhibitory fields enhance visual contrast. Borders and steep intensity gradients in the retinal image are accentuated by maxima and minima (Mach bands) in the response pattern of the optic nerve. A theoretical analysis of the contrast phenomena indicates that the shape of the Mach bands is determined by the configuration of the inhibitory field. Patterns of the optic nerve activity in response to simple, stationary patterns of illumination were measured and compared to theoretically calculated response patterns. The features common to the experimental and calculated response patterns are directly correlated to the most prominent characteristic of the inhibitory field: a diminution in the inhibitory effect near the center of the field. There are, however, some significant discrepancies between theory and experiment resulting most likely from the restriction of the theoretical model to a one-dimensional array of receptors. Preliminary studies using a more realistic two-dimensional representation of the eye are in somewhat better agreement with the experimental results.

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INTRODUCTION

Nervous inhibition in the retina and other sensory systems has received much attention in recent years by students of neurophysiology, psychophysics, and behavior. It is becoming increasingly evident that the interaction of nervious elements and the integration of inhibitory and excitatory influences play an important role in processing sensory information at various levels of the nervous system. The role of nervous inhibition in sensory physiology however is not new. Nearly one hundred years ago Ernst Mach (1865) investigated the long-known ability of the visual system to accentuate contours and borders. With remarkable insight he concluded that the ability must originate in a reciprocal inhibitory interaction of neighboring elements in the retina. More recently, Bekesy (1928) hypothesized a similar mechanism for enhancing frequency discrimination in the auditory system. These speculations based primarily on indirect evidence from psychophysical experiments have since been supported by the direct observation of the responses of single nerve cells located at various levels in the sensory system.

Early evidence on the role of neural inhibition in sensory physiology was obtained by Hartline (1938 and 1940) who recorded complex retinal responses from the optic nerve fibers in the vertebrate eye. He attributed the complexity of the responses to the integrated effects of excitatory and inhibitory influences mediated over pathways that interconnect the ganglion cells and the photoreceptors. A similar observation on the opposed influences in the vertebrate retina was made by Granit (for reviews see Granit, 1947 and 1955). Moreover, neural inhibition has been observed in single auditory nerve fibers (Galambos and Davis, 1944), in higher auditory centers (Suga, 1965), and in the cutaneous system (Mountcastle and Powell, 1959).

In each instance there is strong evidence to suggest that the inhibitory interaction depends on the separation of the elements in the receptor mosaic, which is exactly what Mach postulated to account for the enhancement of visual contrast at contours. If in the visual

system the inhibitory interaction is stronger for near neighbors in the receptor mosaic than for more widely separated ones, then the contrast effects will be greatest in the vicinity of sharp discontinuities in light intensity in the retinal image. That is, certain features of the retinal image such as outlines of objects and edges of shadows will tend to be emphasized at the expense of accurate information concerning the intensity of light at each point in the image. A more accurate description of the visual contrast effects requires a knowledge of the lateral spread of inhibition across the receptor mosaic. In particular, the strength of inhibition exerted by a given receptor on every other receptor within its area of influence must be determined before one can attempt a complete analysis of the enhancement of visual contrast. Several investigators have studied this problem in the vertebrate retina, but their results describe only the very general characteristics of the spread of inhibitory effects. Furthermore, the vertebrate receptive field is usually a combination of excitatory and inhibitory influences, thereby making the analysis even more complicated.

The lateral eye of Limulus polyphemus is an ideal preparation on which to carry out a study of the spatial distribution of the inhibitory interaction. First, the interactions between the receptors are purely inhibitory, and secondly the receptor units in the compound eye are large enough to be illuminated individually so that the strength of inhibition exerted among them can be measured directly. Far more important is that a wealth of information exists on the many aspects of the physiology of this eye. Of particular relevance to this study is the fact that a quantitative analysis of the inhibitory system has been well worked out, while studies of the inhibitory receptive field have been limited to preliminary experiments.

The dissertation to follow is a quantitative experimental and theoretical study of the lateral spread of inhibition in this simple nervous system. A series of experiments has been performed to determine the exact law relating the magnitude of the inhibitory parameters to

the retinal distance between receptors. In addition, a theoretical analysis of the inhibitory system has been carried out to determine the affects of the configuration of the inhibitory receptive field on the enhancement of visual contrast. It was found that given the experimentally measured inhibitory field, the theory will describe some of the general features of the response of the eye to a simple "step" pattern of illumination. Hopefully, the results of this study will lead to a clearer understanding of the processing of visual data by the Limulus eye, and will contribute to the investigation of pattern recognition in other visual systems.

CHAPTER I

THE LIMULUS LATERAL EYE

In 1928 Hartline recorded "small but definite electrical changes" in the optic nerve of Limulus when the eye was exposed to light. Four years later in 1932 using improved techniques Hartline and Graham recorded nerve impulses from single fibers in the lateral eye of this animal. At that time Hartline noted that this eye is an admirable preparation for the study of photoreception. Since 1932 contributions by many scientists have resulted in a wealth of information about the Limulus eye, establishing it as a classical preparation in visual physiology.

The purpose of this chapter is to familarize the reader with the basic anatomical features of the lateral eye and with its important property of mutual inhibition that has been so clearly elucidated by Hartline, Ratliff and their colleagues. It should be emphasized that this chapter constitutes an historical review - the original work of this thesis will be presented in the later chapters.

Anatomy

Limulus polyphemus, commonly known as the horseshoe "crab", is an arachnoid inhabiting the shallow marine waters along the eastern coast of North America. The crab has a lateral pair of compound eyes, a median pair of simple ocelli, and several other photoreceptor structures.* This thesis deals exclusively with the lateral eyes. The following discussion is concerned only with the most general anatomical features of these eyes - for a more detailed description the reader is referred to the histological studies by Miller (1957 and 1958).

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*For recent work on the characterization of these structures see Millechia, Bradbury, and Mauro (1966).

A photograph of the lateral eye is shown in figure 1. The dorsal direction on the eye is toward the top of the page and the anterior direction is to the right. In the adult animal the eye measures 1.0 to 1.5 centimeters across and contains 800 to 1000 sensory units called ommatidia. Each ommatidium measures approximately 250 μ in diameter and is supplied with a crystalline lens that is a part of the cornea. The ommatidia in the center of the eye in figure 1 appear as black discs because their optic axes are oriented in the direction of the camera, that is they absorb light along this axis and scatter it at other angles. The optics axes of adjacent ommatidia diverge so that the visual field of the whole eye covers approximately a hemisphere (Waterman, 1954).

A silver-impregnated cross-section of the compound eye is shown in the micrograph in figure 2. The section is taken perpendicular to the plane of figure 1. At the top of the micrograph are the heavily pigmented ommatidia (the cornea has been removed to prepare the section). Each ommatidium contains a cluster of approximately 12 to 15 cells, one or occasionally two - very rarely three - are eccentric cells, and the rest are retinular cells. The individual cells of the ommatidia cannot be detected in the micrograph due to the heavy pigmentation. However, the nerve fibers that originate in the retinular and eccentric cells are visible. They can be seen emerging from the base of each ommatidium in a densely stained bundle which joins similar bundles from other ommatidia to form the nerve trunk shown at the bottom of the micrograph. The nerve trunk - approximately 10 centimeters long in the adult animal - enters the brain at the optic lobe of the circumesophageal ganglion.

Immediately below the receptor layer small lateral branches of the retinular and eccentric cell nerve fibers form an elaborate network, or plexus, of interconnections. The fine collaterals in the plexus mediate neural interactions among the ommatidia. The type of interaction is purely inhibitory - a feature which, as far as known, is unique to the Limulus eye. The evidence for the inhibitory function

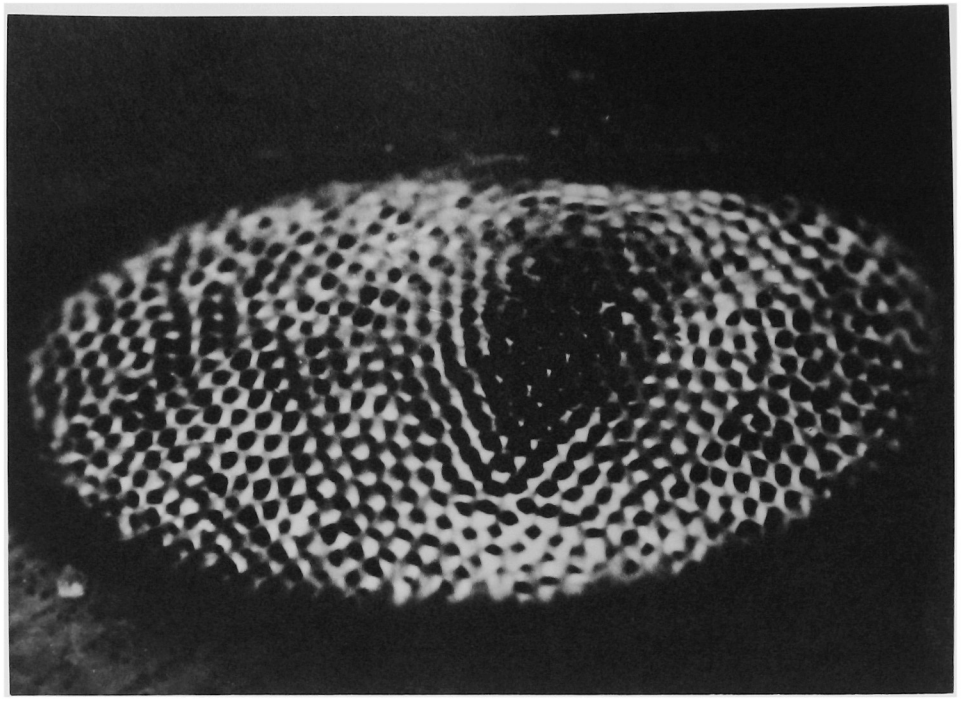

Figure 1. The Limulus lateral eye. The dorsal direction is toward the top of the page and the anterior direction is to the right. Each eye of the adult animal measures about 12 mm long by 6 mm wide and contains 800 to 1000 sensory units called ommatidia. The facets of the ommatidia are spaced approximately 0.3 mm apart, center to center, on the surface of the eye. The ommatidia in the center of the eye appear as black discs because their optic axes are oriented in the direction of the camera. The optic axes of adjacent ommatidia diverge so that the visual field of the whole eye covers approximately a hemisphere. (Photograph prepared by W.H. Miller.)

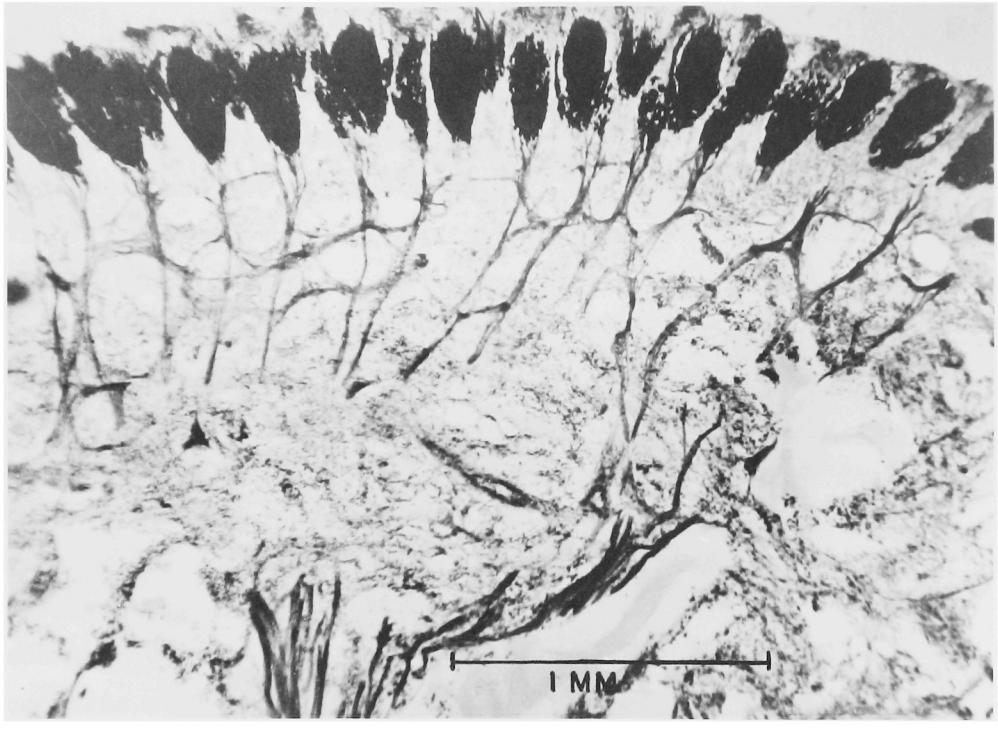

Figure 2. Photomicrograph of a horizontal section of the compound eye of Limulus. The section is taken perpendicular to the plane of figure 1 at a slightly higher magnification. To prepare the section the cornea was removed and the tissue was treated with Samuel's silver stain. At the top of the micrograph are the heavily pigmented ommatidia. The silver-stained nerve fibers originating in the retinular cells and eccentric cells of each ommatidium emerge as a bundle and join with similar bundles from other ommatidia to form the optic nerve shown at the bottom of the micrograph. Immediately below the receptor layer small lateral branches of the retinular and eccentric cell nerve fibers form an elaborate network, or plexus, of interconnections. (Micrograph from Hartline, Wagner, and Ratliff, 1956.)

of the plexus is compelling, even though no neural activity has ever been recorded from it. The most direct evidence was obtained by Hartline, Wagner and Ratliff (1956) who found that cutting the plexus bundles around the strand of nerve fibers from an ommatidium abolished all of the inhibitory effects exerted on it by neighboring ommatidia. A histological study by Hartline, Ratliff, and Miller (1961) showed that throughout the plexus there were numerous clumps of neuropile containing vesicular structures similar to those found in synaptic regions in a wide variety of animals. It is reasonable to suppose that these vesicular structures, found within the eccentric cell branches, as well as in other fibers comprising the neuropile, transmit the inhibitory interactions among ommatidia. That the inhibition is in fact mediated synaptically is indicated by the recording of inhibitory post-synaptic potentials (IPSP) in the eccentric cell body (Hartline, Ratliff, and Miller, 1961; Purple, 1964). Further evidence was obtained by Adolph (1966) who showed that the inhibitory effect could be mimicked with gamma-amino butyric acid (GABA) which has been implicated as the inhibitory synaptic transmitter in the invertebrate nervous system (for a comprehensive review see Curtis and Watkins, 1965).

It is not known, however, if the inhibitory effects are mediated directly by the eccentric cell ramifications or through intervening neurons (interneurons). If the effects are transmitted by collaterals of the eccentric cell axons, then one might expect the same axons to exert inhibition at the central ganglion. On the other hand, the eccentric cell ramifications might exert only excitatory influences which in the plexus would be converted by interneurons to inhibitory influences. The evidence for either influence by the eccentric cell is inconclusive. Nevertheless, it is certain that the plexus is the pathway of lateral inhibition. Before discussing in detail the properties of the inhibitory interactions a few points will be made concerning the function of an ommatidium.

The Ommatidium as a Receptor Unit

Each ommatidium in the lateral eye appears to function as a single "receptor unit". To demonstrate this the optic nerve is first separated into smaller nerve bundles. Then, using the technique of microdissection developed by Adrian and Bronk (1928), one of the smaller bundles is subdivided until a single active nerve fiber remains. By exploring each of the corneal facets in the eye with a small spot of light, the activity in the nerve fiber can be directly correlated with the illumination of one particular ommatidium. Moreover, when recording from a large bundle of active fibers, it is found that the localization of the stimulus to a single ommatidium evokes a discharge of impulses in one and only one nerve fiber in the bundle. Apparently, each ommatidium represents a single receptor unit which can be stimulated only by light entering its corneal facet and which responds to the stimulus by discharging impulses that are conducted to the central ganglion along a single optic nerve fiber.

Hartline, Wagner, and MacNichol (1952) obtained some direct evidence correlating the discharge of impulses in an optic nerve fiber with the electrical activity of an eccentric cell. To do this they impaled the eccentric cell body of a particular ommatidium with a microelectrode and dissected free the bundle of nerve fibers emerging from that ommatidium. Upon stimulation with light they found that large spikes superimposed on the "generator potential" in the electrical record from the eccentric cell were synchronous with the nerve impulses recorded from the bundle of fibers. These results were supported by Waterman and Wiersma (1954) who observed that there was only one active axon associated with each ommatidium and the axon most likely belonged to the eccentric cell. More recently, Behrens and Wulff (1965), and Purple (1964) using combined histological and electrophysiological techniques have shown that the

largest spike potentials in an ommatidium are recorded from eccentric cells. During the course of their study Behrens and Wulff noted several cases where there was a complete absence of spike activity in the recording; subsequent examination of serial sections of the particular ommatidia revealed no eccentric cell. It would appear then, that nerve impulses are discharged only by eccentric cells and are conducted to the brain only by eccentric cell axons.

However, in addition to an eccentric cell, an ommatidium normally contains 11 to 14 retinular cells which also send axons to the central ganglion. What is the function of the retinular cells? The histological studies by Miller (1957, 1958) indicate that the retinular cell bodies contain the photosensitive rhabdoms which presumably communicate information on the intensity of incident light to the eccentric cells. In addition, Ratliff (1966) has shown that local regions of the rhabdom can be selectively light-adapted indicating that the individual retinular cells within a given ommatidium contain particular information on the distribution of light intensity in the visual field. It is not known, however, if they convey this information to nerve cells outside the ommatidium, that is to other ommatidia or to the central ganglion. Certainly the retinular cells with their bona fide axons are well-equipped to do so. The axons, however, have no known function, although they appear morphologically as genuine nerve structures. There have been no reports as yet in the literature of impulses recorded in the retinular cell axons following illumination of the ommatidium. On the other hand, several teams of investigators have obtained evidence that the axons will generate impulses following direct electrical stimulation (Borsellino, Fuortes, and Smith, 1965; Gasser and Miller; Lange and Stevens; the last two are unpublished). Perhaps the axons also generate impulses under natural conditions, but the impulses are too small to detect with the present recording techniques; or perhaps the impulses are discharged in exact synchrony with those from the eccentric cell. Indeed, it is hard to believe that the retinular-cell axons have no function whatsoever. Possibly, future experiments using different recording techniques will provide a

clue to the mystery of the retinular cell.

At this time, however, there is no alternative but to consider the discharge of the eccentric-cell axon as the sole indicator of the propagated response of the ommatidium, that is to consider the ommatidium as a single receptor unit channeling information on incident light intensity to the brain via a single nerve fiber.

Lateral Inhibition

The individual receptor units are not functionally independent. Instead there is a purely inhibitory mutual interaction between neighboring units. Much is known concerning the many aspects of the interaction.* The following is a description of some particular aspects that are relevant to this thesis.

A qualitative description of the inhibitory interaction is shown in figure 3. On the left of the figure are sketches of the eye in cross-section (taken from the micrograph in figure 2). On the right are three oscillograms of impulses recorded from a single fiber which was dissected from the main trunk of the optic nerve (Hartline, Wagner and Ratliff, 1956). The top two records were obtained by focusing a small spot of light on the ommatidium from which the single fiber arises. The intensity of light in the first record is 10,000 times that used in the second. The rate of discharge is roughly proportional to the logarithm of the incident light intensity. The third record was obtained by first illuminating the single ommatidium and then its neighbors. The response of the neighbors (not recorded) produces a concomitant decrease or inhibition of the firing rate of the ommatidium under observation (Hartline, 1949).

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* This subject has been extensively reviewed in several recent publications (Hartline, Ratliff, and Miller, 1961; Ratliff, 1961, 1965; Ratliff, Hartline and Miller, 1963; Ratliff, Hartline, and Lange, 1966; Lange, Hartline, and Ratliff, 1966b).

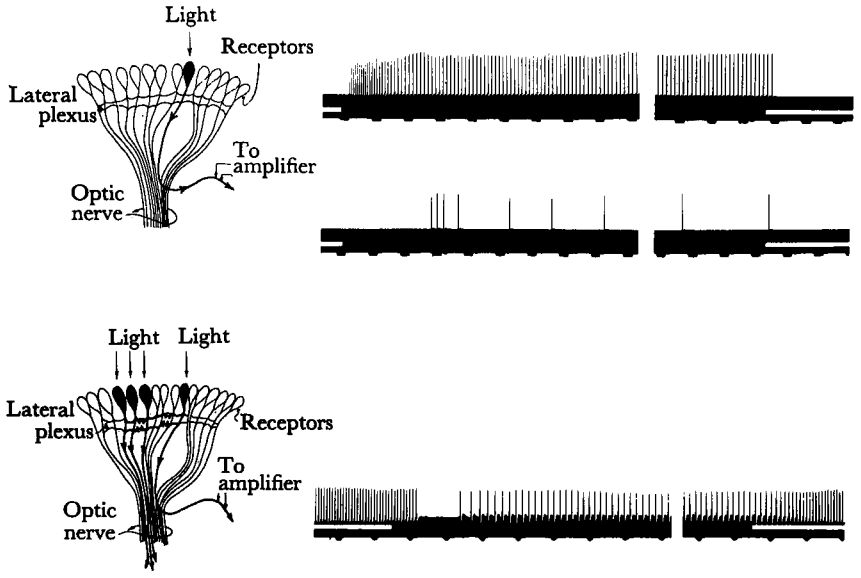

Figure 3. A qualitative description of the inhibitory interaction in the Limulus eye. On the left are sketches of the eye in cross-section indicating the experimental arrangements. On the right are three oscillograms of impulses recorded from a single optic nerve fiber. The top two records show the response to steady illumination of the single ommatidium from which the single fiber arises. The intensity of light in the first record is 10,000 times that used in the second. The duration of illumination is indicated by the blackening of the white line above the 1/5 second time marks. Each record was interrupted for 7 seconds. The third record was obtained by first illuminating the single ommatidium and then its neighbors. The blackening of the white line above the 1/5 second time marks signals the illumination of the neighboring ommatidia. (Top two records from Hartline, Wagner, and MacNichol, 1952; bottom record from Hartline, Wagner, and Ratliff, 1956.)

Before discussing the more quantitative aspects of this inhibitory process, there is one point that needs to be emphasized. Note in the third record of figure 3 that the illumination of the neighboring ommatidia produces a sharp transient decrease in the response of the ommatidium under observation. After the transient the response reaches a steady depressed level. The experiments in this thesis neglect all transient inhibitory effects and deal only with "steady-state" inhibition. The so-called "steady-state" inhibition is defined as the decrease in frequency from a steady uninhibited level to a steady inhibited level. A more precise, operational definition is given in the following chapter. (For a preliminary treatment of the dynamic aspects of inhibition refer to Ratliff, Hartline, and Lange, 1966; Lange, Hartline, and Ratliff, 1966b).

A more quantitative measure of the inhibitory interaction among receptor units can be obtained by recording simultaneously the response of two nearby ommatidia as shown schemmatically in figure 4. (For a detailed description of these classical experiments see Hartline and Ratliff, 1957.) The two ommatidia (A and B) are optically isolated and their nerve fibers are recorded on separate electrodes. The interaction between A and B is determined simply by illuminating A alone, then B alone, and then A and B together. It is found that when A and B are illuminated together they respond at lower rates then when they are illuminated separately. Therefore the inhibitory influences are exerted mutually, that is A inhibits B while B inhibits A. A series of experiments using different light intensities on A and B provides a quantitative measure of the strength of their mutual interaction.

The data from such a series of experiments can be presented in several different ways, but the common observation is that the decrease in the response of one ommatidium is linearly related to the concurrent response of the other. The linearity of the interaction between A and B is shown clearly in each graph on the right in figure 4. The fact

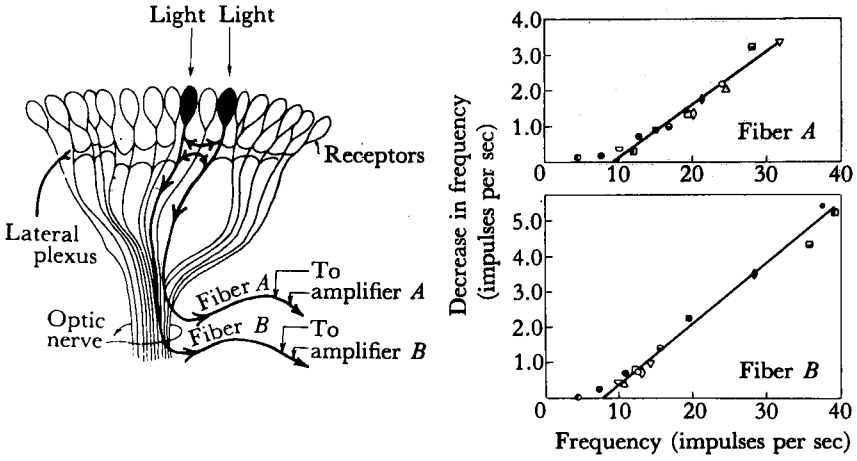

Figure 4. Mutual inhibition of two neighboring receptor units in the Limulus eye. The two receptors (A and B) were optically isolated as indicated in the schematic and their nerve fibers were recorded simultaneously on different electrodes. The interaction between A and B was determined simply by illuminating A alone, then B alone, and then A and B together. In each graph the magnitude of the inhibitory effect (decrease in the frequency of discharge) exerted on one of the receptors is plotted on the ordinate as a function of the concurrent activity (frequency) of the other on the abscissa. The different points were obtained by using various combinations of light intensities on A and B - points with the same symbol indicate data that were obtained simultaneously. The slopes of the lines determine the values of the inhibitory coefficients, K_{AB} and K_{BA}. The intercepts on the abscissa give the values of the inhibitory thresholds, r^{o}_{AB} and r^{o}_{BA}. (Fig. from Hartline and Ratliff, 1957.)

that the inhibitory effects are related to the concurrent responses of the ommatidia and not to the incident light intensities indicates that the inhibition is recurrent.* In neurophysiology, the term recurrent applies to a process in which the response of neurons at a particular level in a nervous system feeds back to affect the response of other neurons at the same level. In the Limulus eye the discharge of impulses from one eccentric cell inhibits the response of other eccentric cells at the point of impulse initiation. In a study of simultaneous brightness contrast in the human visual system Alpern and David (1959) concluded that the inhibitory system in the human eye also has recurrent properties.

Referring back to figure 4 notice that for each ommatidium there is a threshold frequency below which no inhibition is exerted on the other. Above this frequency the relationship is nearly linear. The critical frequency below which there is no inhibition is called the inhibitory threshold.

The main properties of the inhibitory interaction between two units as indicated by the data in figure 4 are: mutuality, recurrence, linearity, and a threshold. These properties can be concisely stated with the use of two simultaneous linear equations:

$$r_A = e_A - K_{AB}(r_B - r^o_{AB})$$

$$r_B = e_B - K_{BA}(r_A - r^o_{BA}) \qquad (1)$$

where the subscripts refer to the respective ommatidia, A and B. In the first equation the response of ommatidium A, r_A, is equal to the frequency of firing of A illuminated alone, e_A, diminished by the inhibitory influence of B. The magnitude of this inhibition is

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* The term recurrent inhibition is used in this context in the same way as it is used to describe the interactions in the spinal cord (Granit, Pascoe, and Steg, 1957; Brooks and Wilson, 1959) and in the hippocampus (Anderson, Eccles, and Loyning, 1963).

expressed by r_B, the concurrent response of B, minus the threshold frequency, r^o_{AB}, that B must exceed before it can inhibit A, and multiplied by the "inhibitory coefficient", K_{AB}. The inhibitory threshold and inhibitory coefficient are labeled to indicate the direction of action: r^o_{AB} is the threshold that B must exceed to inhibit A, and K_{AB} is the inhibitory coefficient for B affecting A. The second equation is the same as the first with the subscripts interchanged to describe the inhibitory effect of A on B.

The parameters in the two equations (1) are correlated directly with the characteristics of the two graphs in figure 4. For example, the values $e_A - r_A$ and $e_B - r_B$ are the decrease in frequency or inhibition of A and B respectively, and correspond to the ordinate in each graph. The parameter e will be generally referred to hereafter as the "uninhibited firing rate" of an ommatidium, that is the resultant of the excitatory influence from its respective light stimulus. As it was mentioned the inhibition is a function of the concurrent - not the uninhibited - firing rate, and therefore the response of the ommatidia, r_A and r_B, are plotted on the abscissa in each graph. The intercepts of the lines describing the data points with the abscissae are the inhibitory thresholds, r^o_{AB} and r^o_{BA}. The slopes of the lines correspond to the inhibitory coefficients, K_{AB} and K_{BA}, which determine the strengths of the inhibitory effects between A and B. For instance, K_{AB} measures the decrease in frequency of A, that is $e_A - r_A$, per impulse of the response of B above threshold. Notice that the inhibitory coefficient and threshold in the upper graph are nearly identical to those in the lower graph. This is the exception and not the rule. The sensitivity of A to inhibition from B may be very different from that of B to A, so that K_{AB} and r^o_{AB} will not necessarily be equal to K_{BA} and r^o_{BA}. It is for this reason that these parameters are labeled with respect to the direction of action.

The values of the parameters in the two equations (1) must obey certain restrictions. The most obvious restriction is that there can

be no negative frequencies, that is the e's and r's must be positive. Another restriction is that the quantity e - r must be positive since the interactions are purely inhibitory. Similarly the K's are also positive. If the terms within the brackets, $r_B - r^o_{AB}$ or $r_A - r^o_{BA}$, lead to a negative value, then they must be replaced by zero. This last restriction is based on experimental fact: an ommatidium that inhibits a neighbor has been found to do so only if its response exceeds a certain threshold value characteristic of the pair and of the direction of action.

The equations, in effect, represent the steady state stimulus response characteristics of two interacting receptor units (ommatidia). The response of each unit is the resultant of the excitatory influence from its respective light stimulus and the inhibitory influence exerted on it by the other unit. When more than two receptors are involved the situation becomes more complex.

However, a representation of the interaction of many ommatidia can be simplied if certain spatial restrictions are observed. Hartline and Ratliff (1958) found that the inhibitory influences from two groups of receptors, that are widely separated on the eye so that the groups do not interact with one another, combine by simple addition when acting together on a common receptor. Thus the total inhibition exerted on a given receptor by all of its "non-interacting" neighbors is merely the sum of the inhibitions exerted by each neighbor individually. Further investigation (Hartline, Ratliff, and Miller, 1961) showed however that the law of the spatial summation of inhibitory effects stated above can be extended to the general case of interacting receptors such that a set of n interacting receptors may be described by a set of n simultaneous linear equations:

$$r_p = e_p - \sum_{\substack{j=1 \\ j \neq p}}^{n} K_{pj}(r_j - r^o_{pj}) \qquad (2)$$

where p = 1, 2, ...n. The subscript p can represent any given receptor, and the subscript j then refers to any of its n-1 neighbors.

The restrictions that were just outlined for the preceding pair of equations (1) also apply to the set of equations above: no negative frequencies - e and r must be equal to or greater than zero, the K's are positive, and the differences $(r_j - r^o_{pj})$ must be set equal to zero whenever r_j is smaller than r^o_{pj}. The equations are therefore "conditionally" linear, that is they are linear only within the regions defined above. This is true only to the first approximation. Recent experiments by Lange (1965) and the author (Appendix I) indicate that K is dependent upon e. To include this in the formal description of the inhibitory system requires a next order correction of equation (1). As pointed out in Appendix I the correction introduces a non-linearity which may be significant to the visual perception of the animal, especially at low levels of incident illumination.

The requirement $j = p$ indicates that the summation does not consider the possible inhibitory effect of a receptor upon itself due to its own activity. This so-called "self-inhibition" has been defined by Stevens (1964) in terms of the response of a receptor to sudden changes in the intensity of stimulation. Briefly, increments and decrements in the incident light intensity are accentuated in the receptor's response. These effects - first noticed by MacNichol and Hartline, 1948 - have since been attributed by Stevens to self-inhibition. These effects, however, express certain mechanisms operating within the ommatidium itself and consequently are not relevant when the ommatidium is considered as a functional unit. Therefore the self-inhibitory effects are excluded from the summation in (2) and also from the theoretical treatment in Chapter IV.

The Lateral Spread of Inhibition and the Enhancement of Contrast

The inhibitory influences among ommatidia diminish with increasing distance between them. The experimental results in figure 5 (Ratliff and Hartline, 1959) indicate that the inhibitory coefficient (slope) decreases with distance while the threshold of inhibition (x-intercept) increases. Notice that the coefficient of inhibitory action of A on B is greater than that of A on C. The variation of the inhibitory threshold shows that A must respond at a higher firing rate to inhibit its distant neighbor C than to inhibit its near neighbor B. These "distance effects" can be readily incorporated into the existing set of equations (2) without adding new terms: the diminution of the inhibitory influence with distance may be ascribed simply to the combined effects of decreasing the inhibitory coefficients (K_{pj}) and increasing the inhibitory threshold (r^{o}_{pj}).

The ability of neighboring receptors to exert greater mutual inhibition than more widely separated ones leads to characteristic contrast phenomena. Ratliff and Hartline (1959) showed that the patterns of optic nerve activity are not direct copies of the patterns of external stimulation, but rather the borders and contours in the visual image are accentuated. Brightly illuminated receptors located near a discontinuity in the visual image will inhibit those neighbors on the dimly illuminated side by a greater amount than the neighbors in weak light will inhibit them, thereby enhancing the discontinuity in the neural response pattern. Ratliff and Hartline pointed out that inhibitory influences which diminish with retinal separation will accentuate contrast at borders and steep intensity gradients in the visual image.

Many years ago Mach (1865) arrived at a similar conclusion in his study of contrast phenomena in the human visual system. The light and dark bands seen at borders and steep intensity gradients in the retinal image led Mach to conclude that both phenomena

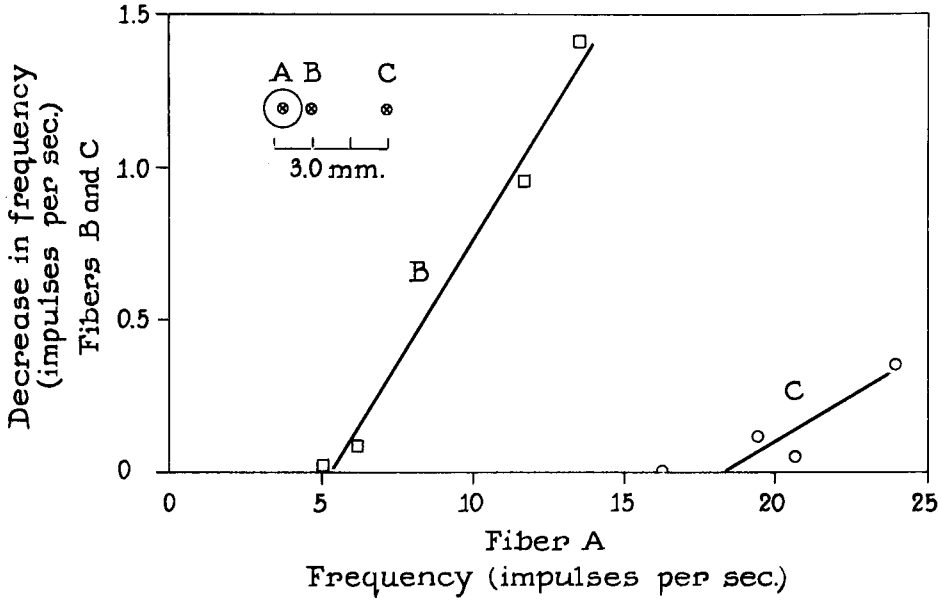

Figure 5. The diminution of the magnitude of inhibition with distance. The inhibition (decrease in frequency) exerted by a small group of receptors (A) on two other receptors (B and C) is plotted on the ordinate. The concurrent frequency of the impulse discharge from one receptor in the center of the group A is plotted on the abscissa. The configuration of the pattern of illumination on the eye is indicated by the insert. The dots represent the facets of the receptors whose responses were recorded. Receptor A was located in the center of a group of six or seven receptors illuminated with a spot of light 1 mm in diameter. Illumination of the near (B) and distant (C) neighbors was provided by spots of light 0.2 mm in diameter. The inhibitory effects of group A on B and on C were determined separately. The strength of the inhibitory action is measured by the slope of the line (inhibitory coefficient) and the threshold of the action is measured by the x-intercept (inhibitory threshold). (Figure from Ratliff and Hartline, 1959.)

originate in the mutual inhibitory influence of neighboring receptors. He hypothesized that the influence was mediated with a diminishing effect over the lateral network of neural interconnections in the retina. In recognition of Mach's clear insight into the physiological foundation of visual contrast phenomena, the light and dark bands have been named Mach bands.*

In both the human and Limulus visual systems the enhancement of contours and borders, in effect, distorts the retinal image: certain features of the spatial distribution of light intensity are enhanced at the expense of accurate information on the intensity of stimulation of each receptor. The subjective image therefore is not directly correlated with the physical reality of the surround. Instead the eye is "tuned" to particular characteristics of the visual field, namely edges and contours that are highly contrasted either by their natural properties or by artifical highlights and shadows. For the Limulus this selective property may be of primary importance to its feeding and breeding behavior; unfortunately very little is known concerning the visual stimuli that confront the animal in its natural habitat. For the human this property not only dictates the objects that catch our eye but also influences to a great extent our creative expressions in art and architecture. Whatever the consequences may be it is clear that the eye selects particular information from the immense detail in the visual image, enhances it at the expense of less significant information, and then transmits this modified image to the central nervous system.

As yet an exact theoretical treatment of this selective property cannot be given for the Limulus eye, the human visual system or any other visual system because the exact law relating the magnitude of the inhibitory influence to the retinal distance between receptors

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* Mach bands will be discussed in greater detail in Chapter IV. For a thorough study of the Mach bands and their significance refer to Ratliff's book (1965).

is not known. Based on the previous observations by Ratliff, Hartline, and Mach it is evident that a sufficient condition for contrast enhancement is that the inhibitory influence diminish with increasing distance on the retina. Kirschfeld and Reichardt (1964) derived theoretically the spatial distribution of the inhibitory influence in the Limulus eye from a consideration of the shape of Mach bands. The variability of their experimental data however casts some doubt on the validity of their conclusion (Reichardt, personal communication).

A more straight-forward approach to this problem is to measure the distribution of the inhibitory influence directly on the retinal mosaic. That is to fill in the gaps of the experiment in figure 5 by measuring the inhibitory coefficients for the ommatidia between B and C and for as many ommatidia surrounding A as possible. Knowing the coefficients for all points around A would constitute a map of the inhibitory field of A. This would be equivalent to measuring each K_{pj} in the set of simultaneous equations (2). In addition the distance function of the inhibitory threshold, r^{o}_{pj}, would also be determined. Knowing these two parameters for every value of p and j in a two-dimensional array of elements would allow an exact theoretical treatment to be carried out.

The goal of the experiments in this thesis is to determine the law relating the inhibitory parameters to the retinal separation of receptors. To do this a fiber optics stimulation system was constructed to provide a convenient and accurate method of illuminating one-by-one many ommatidia during the course of a single experiment. The fiber optics system is described in detail in the following chapter. After presenting the experimental data from the mapping experiments (Chapter III) an attempt is made to treat theoretically the Mach band phenomena using a model system based on the set of simultaneous linear equations (2).

CHAPTER II

METHODS

Biological Preparations

The lateral eye together with a short length (1 cm.) of optic nerve is removed from an adult Limulus measuring about 20 cm. across the carapace. The carapace surrounding the eye is trimmed to fit the black lucite chamber shown in the center of figure 6. The bracket supporting the chamber allows the chamber to be rotated from the horizontal position for dissection of the optic nerve to the vertical position for location of the fiber optic light sources (see figure 11a). After mounting the eye in the chamber with melted beeswax, the chamber is rotated to the horizontal position and filled with artificial sea water (Instant Ocean by Aquarium Systems, Inc.). Small bundles of fibers and single fibers are dissected from the optic nerve with fine glass needles. The nature of the dissection depends on the experiment to be performed. Chapters III and IV discuss further the dissections required for the various experiments in those chapters.

At this point in the dissection it is standard procedure to test the eye for the presence of inhibition. One of the dissected nerve fibers is placed on a wick electrode which is connected through a preamplier to an oscilloscope. The ommatidium whose optic nerve is being recorded is illuminated with a fiber optic light source. Probing nearby ommatidia with another light source, the experimenter can easily detect the presence of inhibition either by observing the pattern of impulse discharge on the oscilloscope or by listening to the pattern of discharge over a loudspeaker which is connected to the oscilloscope. If the eye responds abnormally, and strong inhibition is not found, then the preparation is discarded. Otherwise the dissection is continued. When the desired dissection is obtained, the chamber is covered and sealed with paraffin. Throughout the

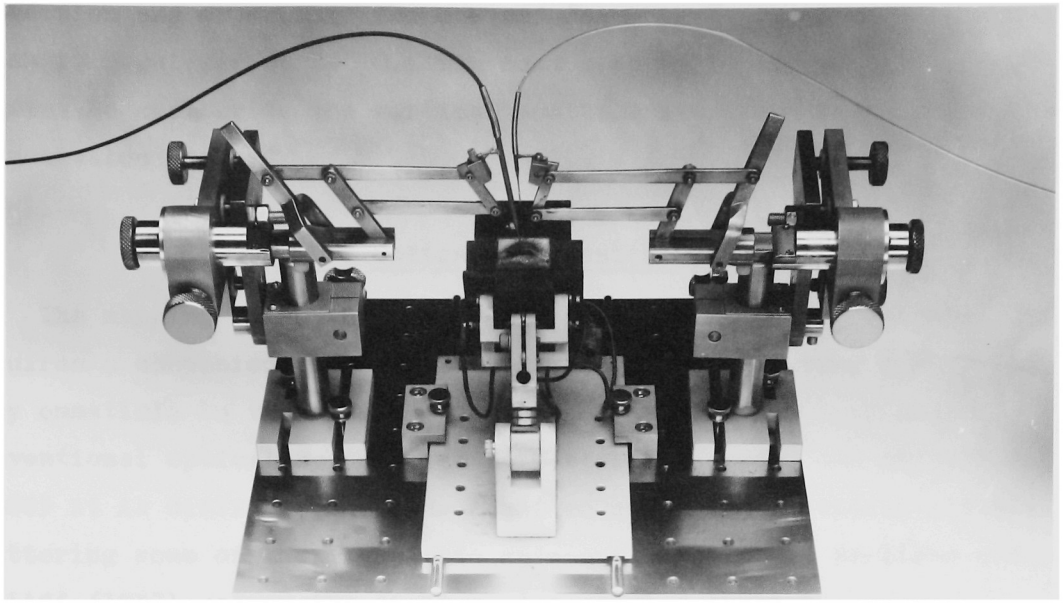


Figure 6. A typical experimental setup employing the fiber optics illumination system. In the center an excised Limulus eye is mounted in a black lucite chamber which contains electrodes for recording activity from optic nerve fibers behind the eye. The hinged bracket supporting the chamber allows the chamber to be rotated to various positions to accommodate the particular experimental situation (see Figure 11a). On either side of the chamber are manipulators that were designed especially for locating optical fibers on the lateral eye. The fibers seen as two curved "lines" - are securely connected to the manipulators. On the right is a single optical fiber and on the left is a fiber bundle (see text). The various adjustments on the manipulators provide the necessary degrees of freedom for aligning the optic axis of an optical fiber with the optic axis of an ommatidium (see Figure 11b). The chamber and manipulators were designed by H.K. Hartline with modifications by the author and constructed by the Instrument Shop at Rockefeller University. The optical fibers were made by the American Optical Company, Southbridge, Mass.

dissection and experiment the eye and chamber remain at room temperature which is regulated at 18° C. The next step in the preparation is to rotate the chamber to the vertical position and align the fiber optics illumination system.

Fiber Optics Illumination System

The mapping of inhibitory fields in the Limulus lateral eye requires a convenient and accurate method of illuminating one by one many ommatidia in the course of a single experiment. With the conventional optical system used by Hartline, Wagner, and Ratliff (1956) it is difficult to focus light on a single ommatidium without scattering some of the light into adjacent ommatidia. Hartline and Ratliff (1957) solved the problem of scattered light by

> "coating the eye with opaque wax (a heavy suspension of lampblack in paraffin wax) and then removing the coating carefully from a small region, exposing the corneal facet of just that one ommatidium from which it was desired to record impulses. The black wax evidently prevents internal reflections inside the cornea of the eye, for by this method perfect isolation of single units can often be obtained, - a result rarely achieved merely by focussing a small spot of light on the facet by means of a lens."

Using this technique Dr. Hartline and his colleagues determined many of the important aspects of mutual inhibition. However, the technique is not practical for experiments that require the illumination one by one of many ommatidia without light scatter. A completely different approach to the problem of scattered light was made with fiber optics.

a) General Description

"Fiber optics" utilizes the property of total internal reflection to conduct light down long thin fibers of glass. For a detailed description of the properties and applications of fiber optics refer to the book by Kapany (1967). The fibers, often called light pipes, are flexible and they can be made with diameters as small as 25 μ. Optically, the fiber is composed of two parts: the core and the

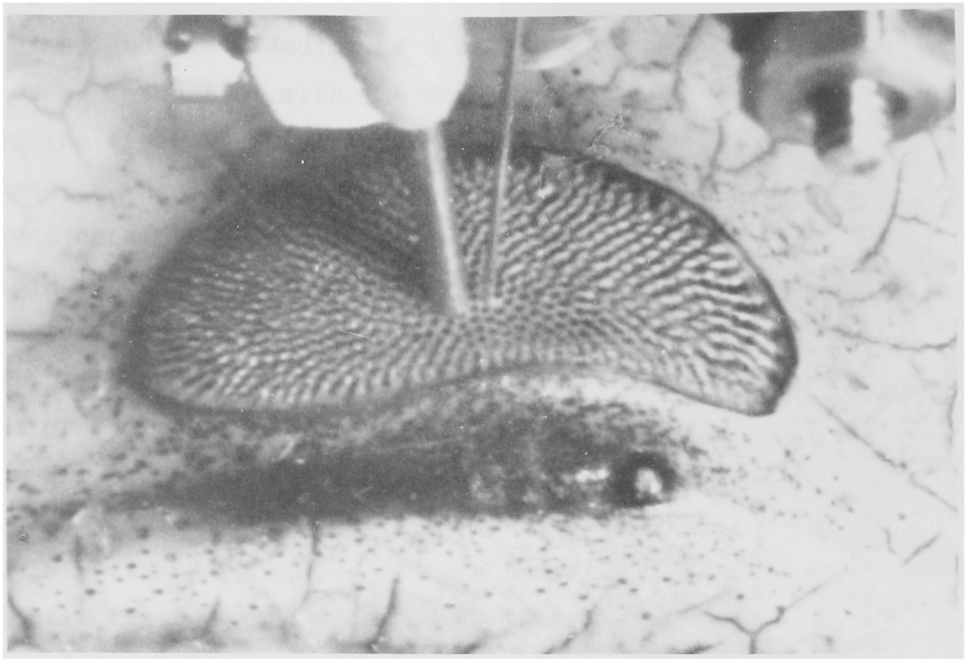


Figure 7. A close-up of the experimental setup in Figure 6. Located on the eye are the two fiber optic systems that are normally used together to map the ommatidial inhibitory fields. For protection from breakage each one is encased in hypodermic tubing. On the right is the single optical fiber which is 70 μ in diameter (encased in 250 μ diameter tubing). Using the proper techniques the light emerging from the single fiber can be contained within the facet of a single ommatidium. The instrument on the left is a bundle of 31 single fibers packed in hypodermic tubing. When placed in contact with the cornea the bundle of fibers illuminates an area (500 μ in diameter) containing from four to six ommatidia.

cladding. The core is a thin, solid rod of glass with a refractive index of n_1 and the cladding is a sleeve of glass with a refractive index of n_2 in contact with the core. Light is conducted down the core of the fiber by total internal reflection at the core-cladding interface, $n_1 > n_2$, and emerges from the tip of the fiber in a solid divergent cone. Figure 8 is a diagrammatic drawing of the tip of the fiber in cross-section showing the critical angle of reflection between the core and cladding and the maximum divergent light ray. With simple geometrical optics it can be shown that the refractive indices of the core, cladding, and medium determine the angle of the maximum divergent light ray by the formula $n_o \sin\Theta = (n_1^2 - n_2^2)^{\frac{1}{2}}$. The value of $n_o \sin\Theta$ measures the light collecting power of a fiber and, hence, is referred to as the fiber's numerical aperture. All of the fiber optic instruments described in this chapter were made by the American Optical Company, Southbridge, Mass. Each instrument is assembled from one or more standard fibers with the specifications listed in Table I. It should be noted that in Table I the acceptance angle (2Θ) - which determines the maximum divergent light rays that are transmitted by the fiber - is equal to the divergent cone of light that is emitted by the fiber.

b) Single Fiber Instrument

The technique used throughout this thesis for illuminating single ommatidia employs a single fiber instrument. The instrument is made by inserting a three-foot length of standard optical fiber into an equivalent length of stainless steel hypodermic tubing. The ends are embedded in epoxy resin and then ground and polished to a fine optical surface. The flexible hypodermic tubing supports the fiber during the grinding and polishing operations and protects the fiber from breakage during normal usage. The tip of the instrument is shown in contact with the Limulus eye on the right in figure 7 which is a close-up of figure 6. It is apparent from figure 7 that the diameter of the hypodermic tubing (250 μ) is approximately equal to the diameter of an ommatidium in an adult eye. The diameter of the single fiber inside

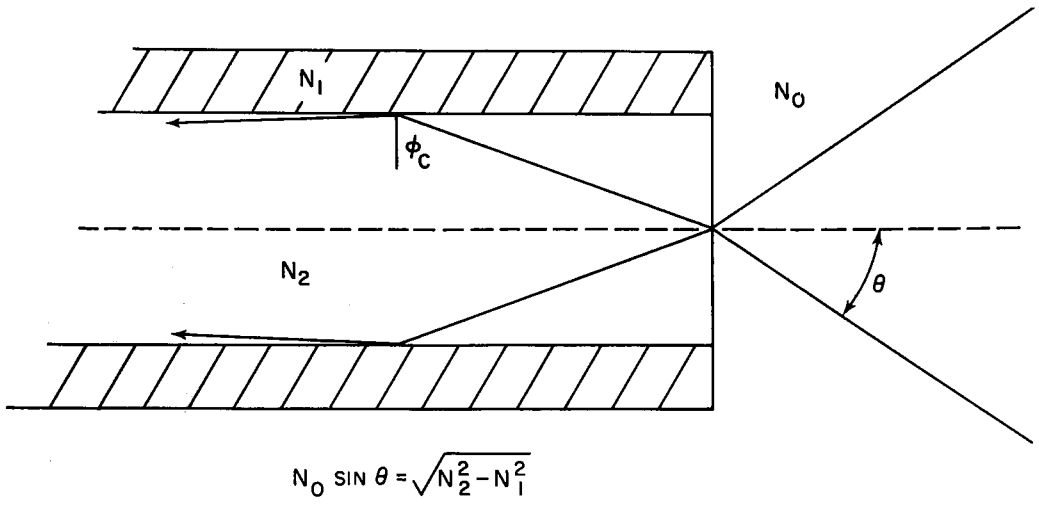


Figure 8. A diagram of the tip of a glass fiber illustrating its optical properties. The fiber is composed of two parts: the core of refractive index n_2 and cladding (cross-hatch) of index n_1. The refractive index of the surrounding medium is n_o. The dashed line indicates the optic axis of the fiber. The solid lines (with arrows) represent the maximum divergent light rays which are transmitted by total internal reflection down the core of the fiber. Light rays that enter the tip of the fiber at angles greater than θ will exceed the critiral angle φ_c at the core-cladding interface and will not be transmitted by the fiber. The refractive indices of the core, cladding, and medium determine the angle θ by the formula $n_o \sin\theta = (n_1^2 - n_2^2)^{1/2}$ where $n_o \sin\theta$ is the fiber's numerical aperture. The same analysis applies to the light rays emerging from the other end of the fiber.

Table I

Fiber Diameter	76 μ
Refraction Index	
Core (flint glass) N_2	1.62
Cladding (crown glass) N_1	1.52
Acceptance angle (2θ)	
Air (N_o = 100)	68^o
Mineral oil (N_o = 1.48)	44^o
Light loss	
Ends	30%
Transmission	10%/foot

Specifications of the standard optical fiber used in the construction of the single fiber instrument and in the fiber bundle. The standard fiber is manufactured by the American Optical Company, Southbridge, Mass.

the tubing is 76 μ or approximately one-third the diameter of an ommatidium.

To illuminate a single ommatidium the fiber is brought into contact with the cornea directly in front of the ommatidium (see figure 7). The optic axis of the fiber is aligned with the optic axis of the ommatidium for maximum sensitivity (Waterman, 1954). The diverging cone of light illuminates no more than one ommatidium. Normally about one-third to one-half of the cornea is removed with a razor blade to decrease the optical path from the fiber to the receptor layer. Most of the lens structure of each ommatidium remains intact after shaving and assists in the optical isolation by partially refracting the cone of light from the fiber (Makous, 1964). The application of mineral oil between the tip of the fiber and the corneal surface further assists the optical isolation by decreasing the cone of light 35 percent.

> The index of refraction for air is 1.00 and for mineral oil is 1.48. From the equation for the numerical aperture, $n_o \sin\Theta = (n_1^2-n_1^2)^{\frac{1}{2}}$, 2Θ equals 68^o for air and 44^o for mineral oil.

The simple operations of shaving the cornea and applying mineral oil guarantee the complete optical isolation of single ommatidia at moderate intensities of illumination.

Visual proof of this statement is given in the photograph in figure 9. This picture, taken through a dissecting microscope, shows the single fiber instrument illuminating an ommatidial lens facet. The eye was sectioned in a plane parallel to the ommatidial optic axis, and the receptor layer was peeled from the cornea exposing each lens cone. A "single fiber instrument" is shown in contact with an unshaven and unoiled cornea illuminating a single facet with the maximum light intensity obtainable from the illumination system to be described in the following paragraphs. The brightness of the facet is due to light scatter at the partially cut surface of the crystalline cone. In an intact facet the incident light is brought to a focus near the tip of the cone without significant loss due to scattering.

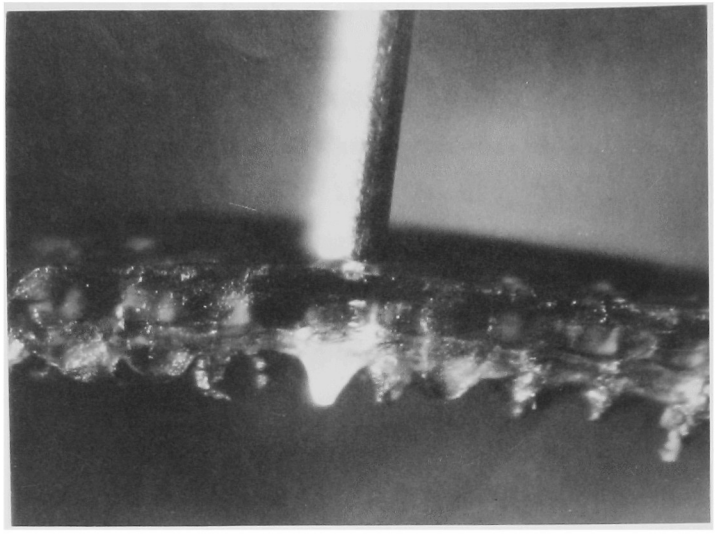


Figure 9. A visual demonstration of the optical isolation of a single ommatidium with an optical fiber. The eye was sectioned in a plane parallel to the ommatidial optic axis, and the receptor layer was peeled from the cornea exposing each lens cone. The view is approximately tangential to the corneal surface facing the cut edge. The single fiber instrument - coming in from the top of the picture - is in contact with the cornea. Light from the dissecting lamp is reflected by the stainless steel tubing of the instrument. Most of the light emitted by the glass fiber (inside the tubing) is contained within a single lens facet.

Moreover, in the intact eye any light that is not directed toward an ommatidium is absorbed by a heavily pigmented sheath that surrounds the crystalline cone protruding from the cornea. For an experiment the light intensity normally is 1,000 to 10,000 times less intense than maximum. Notice in figure 9 that most of the light from the fiber is contained within a single lens facet. The quality of optical isolation can be improved by shaving off some of the cornea and applying mineral oil to the cut surface.

A word of caution: complete optical isolation should not be taken for granted especially at high intensities of illumination (see Appendix II). Usually the quality of optical isolation can be monitored throughout an experiment. For example, in experiments that map inhibitory fields many ommatidia are being recorded simultaneously (see Chapter III). Therefore, each ommatidium can monitor the optical isolation of its neighbor and visa versa. The breakdown of optical isolation is detected immediately by the appearance of spurious nerve impulses on the recording apparatus. It is conceivable that low-intensity scattered light too weak to initiate impulses in neighboring ommatidia may alter experimental results. This important point was investigated with the result that scattered light was shown not to be significant. The results are discussed in detail in Chapter III.

The intensity of light transmitted by a single fiber depends on the method of illuminating the end of the fiber. The most efficient method is to match or exceed the fiber's numerical aperture with the numerical aperture of the illumination system.

> The criterion is satisfied by inserting a 45x microscope objective, N. A. = 0.65, between the light source and the fiber, N. A. = 0.56. The light source is a low-voltage D. C. regulated tungsten filament lamp (G.E. No. 1493, 6 volts, 2.8 amps). A field lens focuses the filament on the back end of a 45x objective that de-magnifies the image of the field lens on the tip of the fiber. The light beam is interrupted by an electro-magnetic shutter (Hartline and McDonald, 1947) and neutral density filters (Kodak Wratten filters) that control the intensity of illumination of the fiber.

With this system the maximum intensity of light transmitted by the single fiber in the 400 mμ to 650 mμ region is 2.4×10^{14} quanta sec^{-1}.

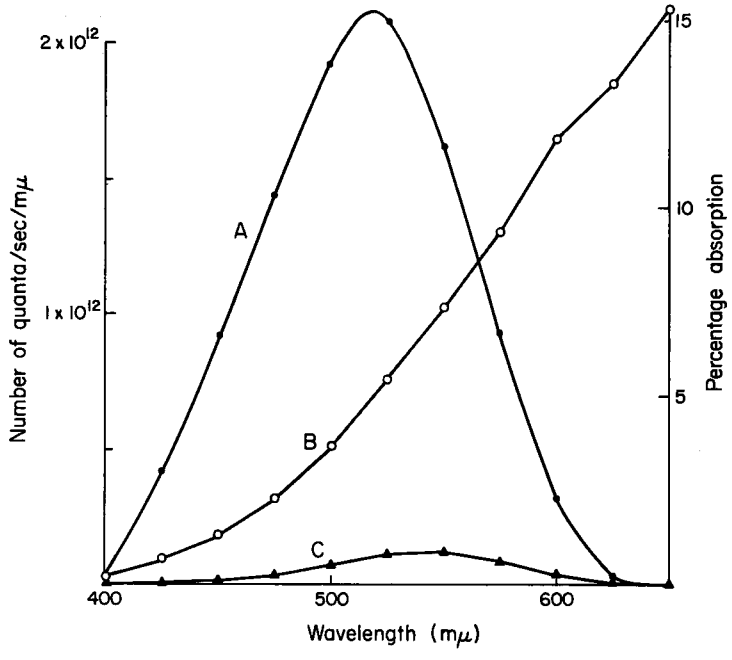
> Curve B in figure 10a gives the spectral characteristics of the light transmitted by the single fiber when it is illuminated with maximum intensity by the system just described. The transmission curve was measured with a model SR Spectroradiometer made by Instrumentation Specialties Co., Inc., Lincoln, Neb. Integrating the area under curve B gives the total power output of the fiber from 400 mμ to 650 mμ equal to 2.4×10^{14} quanta sec^{-1}. Curve A in figure 10a is the absorption spectrum of Limulus rhodopsin in solution calculated from the extinction measurements made by Hubbard and Wald (1960). Hubbard and Wald estimated that the percentage absorption of the visual pigment in situ is 15% at 520 mμ. This estimate assumes that Limulus rhodopsin, like that of other animals, has a molar extinction of about 40,000 and that the 4×10^{-6} μmole of rhodopsin in each eye is evenly distributed over the photosensitive rhabdom regions in the retinula cells. Multiplying curve B by curve A gives the intensity of light from 400 mμ to 650 mμ that would be absorbed by an ommatidium if the incident light were concentrated on the rhabdom regions. Curve C in figure 10a is the product of curve A and curve B. Integrating the area under curve C, replotted on an expanded ordinate in figure 10b, gives 1.3×10^{13} quanta sec^{-1}.

With optimal illumination from a single fiber the maximum intensity of light absorbed by an ommatidium is 1.3×10^{13} quanta sec^{-1} or about 5 percent of the incident light. Experiments described in Appendix II suggest that incident light intensities equal to or greater than 10^{14} quanta sec^{-1} are beyond the physiological range of the ommatidia in the Limulus eye.

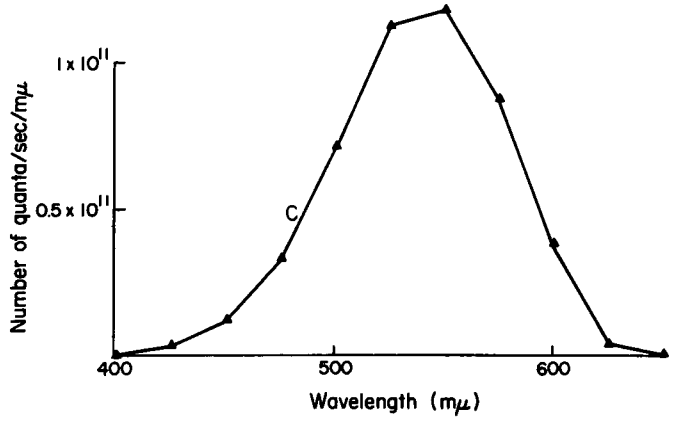
c) Fiber Bundles

Packing a number of optical fibers in one bundle provides an efficient method for illuminating small clusters of ommatidia. Particularly useful is the LGM-1 fiber bundle produced by the American Optical Company. The bundle contains 31 standard fibers (see Table I) packed in a hexagonal array and sealed at both ends with epoxy inside short lengths of hypodermic tubing. The tip of the bundle is shown in contact with the eye on the left in figure 7.

Figure 10. The spectral characteristics of the light transmitted by a single optical fiber and that absorbed from the fiber by the visual pigment in the ommatidia of the Limulus eye. In Figure A curve B gives the quantum output of the optical fiber when the end of the fiber is maximally illuminated with a tungsten filament light source. Curve A is the absorption spectrum calculated from the extinction measurements by Hubbard and Wald (1960) of the visual pigment in the ommatidium. The product of curve A and curve B gives curve C which represents the maximum amount of light (400 mμ to 650 mμ) that an ommatidium could absorb if the incident light from the fiber were concentrated on the photosensitive area of the ommatidium. Figure B replots curve C on an expanded scale. Integrating the area under curve C gives 1.3×10^{13} quanta/sec. which represents 5 percent of the total incident light (area under curve B).


2 x 10^12
1 x 10^12
Number of quanta/sec./mμ
15
10
5
Percentage absorption
A
B
C
400
500
600
Wavelength (mμ)

A


1 x 10^11
0.5 x 10^11
Number of quanta/sec/mμ
C
400
500
600
Wavelength (mμ)

B

The 31 tightly packed fibers occupy an area with a diameter of 500 μ. The grain of the individual fibers is not resolved by the Limulus eye. The number of ommatidia illuminated by the fiber bundle placed in contact with the cornea depends on the position of the bundle with respect to the hexagonally packed ommatidia. The fiber bundle can be positioned to illuminate completely four ommatidia or to illuminate completely only one ommatidium and partially illuminate its six nearest neighbors. To facilitate the calculation of inhibitory coefficients the bundle is normally situated to illuminate four ommatidia. The fiber bundle requires the same illumination system as the single fiber (see preceeding section).

The single fiber instrument and the fiber bundle are used together to map inhibitory fields as described in Chapter III.

d) Mach Band Instrument

The complex nature of the inhibitory field in Limulus eye generated much interest in the response of the eye to patterned light stimulation (see Chapter IV). Ratliff and Hartline (1959) measured the response of the eye to a step pattern of light intensity projected on the eye by focused optics. Due to inherent limitations in the focused optics system the projected image of the step pattern illuminated less than 10% of the ommatidia in the eye. To avoid "edge effects" (see Chapter IV) the area of illumination must be increased. One possible solution is to place a large bundle of optical fibers on the cornea of the eye that will intercept the combined visual field of the ommatidia beneath it and, thereby, be an efficient method for illuminating large areas on the eye. With this in mind I designed a fiber optic instrument that would illuminate approximately 80% of the ommatidia in an adult eye with a step pattern. At the eye the instrument emits light from two adjacent fiber bundles separated by a 25 μ metal partition. Each bundle measures 4.8mm x 4.8mm and is tightly packed in a square array with approximately 216,000 fibers.

> The instrument was constructed by the American Optical Company from Multifibers which are prefabricated bundles each containing 36 square fibers. Each square Multifiber measures 60 μ x 60 μ and each of the 36 fibers in the Multifiber measures 10 μ x 10 μ. There are approximately 6,000 Multifibers or 216,000 single fibers in each half of the Mach Band instrument giving a total of 432,000 fibers for the whole instrument.

The bundles, each three feet in length, are held together at the common end with epoxy and the entire area (0.6mm x 4.8mm) is ground and polished to an optically clean surface. The two bundles bifurcate a short distance from the common end and connect to separate light sources.

> Light sources for the Mach band instrument must be capable of illuminating the entire end of each bundle with a cone of light having a numerical aperture of 0.5 to 0.6. Two American Optical K-150 Illuminators with Sylvania DCL projection lamps are adequate for this purpose. The cone of light from the parabolic mirror in the projection lamp matches the numerical aperture of the fibers in the Mach band instrument and completely illuminates the end of each bundle. Each Illuminator is fitted with an electromagnetic shutter and filter holder, and the lamps are powered by a 120 volt regulated D.C. power supply.

e) Rigid Optics vs. Fiber Optics

The results from some preliminary experiments on the Limulus eye suggested that the fiber optics system might be more effective than the rigid optics system for illuminating single ommatidia. This is not so. To test this point the fiber and rigid optic systems were calibrated over a 1000-fold range of light intensities by a photodiode (Type 1N2175 from Texas Instruments, Inc.).

When properly adjusted for equal light intensities at the cornea both systems produce the same frequency of impulse discharge from an ommatidium. However, the superior optical isolation by the fiber optics system permits it to be used at higher light levels than the rigid optics system. In this respect the fiber optics system is "more effective" than the rigid optics system.

The only difference between the two illumination systems that could be detected by the eye is the size of the cone of light entering the cornea. The cone of light from a single fiber is 68° in air and 44° with oil immersion. From the rigid optics system the cone is 33° with a high power condensing lens and 14° with a lower power lens. (To prevent light scatter when using the rigid optics system a 250 μ aperture was placed in contact with the eye.) An ommatidium will not discriminate between a cone of light as large as 68° and one as small as 14° when the total light flux in each cone is the same. The reason for this has not been established. Apparently there exists in each ommatidium a mechanism that compensates for the limitations introduced by the fixed lens system. Just as the fixed receptor layer in a higher order vertebrate eye requires a variable lens, the fixed lens of a compound eye requires a variable or elongated receptor layer. The elongated rhabdom structure in each ommatidium of the Limulus eye may be the mechanism that enables an ommatidium to utilize light that passes through the cornea irrespective of the size of the cone (within limits).

f) Manipulators

On either side of the chamber in figure 6 are manipulators that were designed by Dr. H.K. Hartline for locating light pipes on the Limulus eye. Also included in figure 6 is a single fiber and a fiber bundle which are securely connected to the manipulators. The design of the manipulators is based on a pantagraphic principle that provides the five degrees of freedom necessary for aligning the optic axis of the light pipe with the optic axis of an ommatidium (refer to figure 11b). Three coordinates are required to locate a point in space and five coordinates are required to locate a vector in space. In this case the optic axis of a light pipe can be considered a vector. In practice the x, y, and z adjustments on the manipulator move the tip of the light pipe into position above the ommatidium to be illuminated. The the optic axis of the light pipe is lined up with the optics axis of the ommatidium by the two rotational adjustments (θ, φ) on the manipulator.

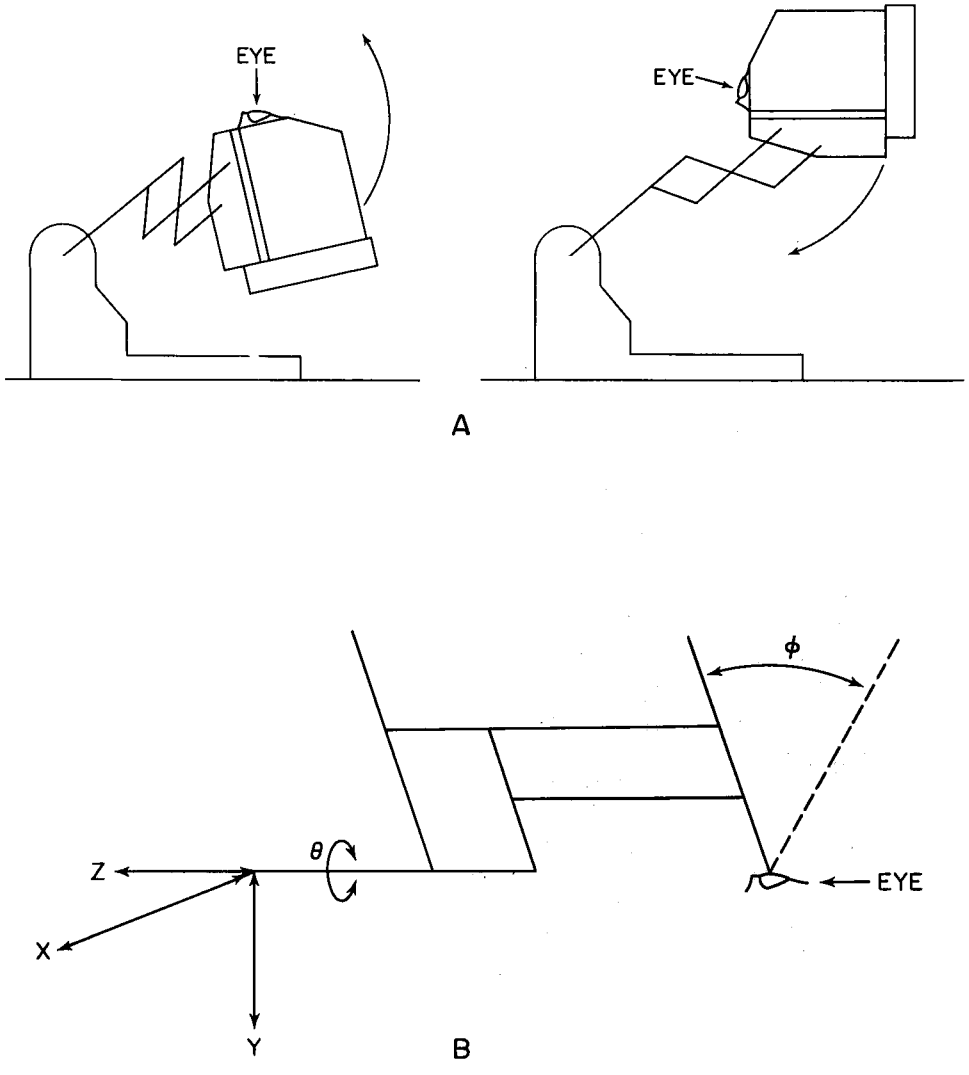


Figure 11. The various adjustments of the experimental chamber and manipulator for locating optical fibers on the lateral eye. The design of both instruments is based on a pantagraphic principle. The diagram in Figure A is a side view of the black lucite chamber shown in Figure 6. Rotating the chamber from the horizontal position (for dissection) to the vertical position exposes the eye in full view to facilitate the location of the fiber optics. The various adjustments on the manipulator are illustrated in Figure B. The three translational adjustments (x, y, and z) are used to locate the fiber (indicated by the solid line touching the eye) above the particular ommatidium to be illuminated, and then the two rotational adjustments (θ, ϕ) are used to align the optic axis of the fiber with that of the ommatidium.

Data Collection and Processing

For most of the experiments in this thesis the raw data consists of many trains of nerve impulses recorded from one or more optic nerve fibers. Sophisticated methods for collecting and processing these data have been developed by Drs. David Lange, H.K. Hartline, Floyd Ratliff, Robert Schoenfeld, and Norman Milkman. In brief, a computer, a programmed timer and associated equipment are integrated to control and monitor an experiment, and to collect, preserve and process the data. The following is a summary of these methods. For a more detailed description the reader is referred to Dr. Lange's thesis (Lange, 1965) or publication (Lange, Hartline, and Ratliff, 1966a).

> A single nerve fiber or a small bundle of nerve fibers is placed on a cotton wick electrode. The electrode is connected through a high input impedance preamplifier to an oscilloscope which amplifies the nerve impulses and feeds the amplified signal to an audio system and to an electronic discriminator. The discriminator will signal the arrival of a nerve impulse when the peak voltage of the impulse exceeds a preset level. The signal from the discriminator is sent by way of a digital converter (Schoenfeld, 1964) to a digital computer (Control Data Corporation 160 A). The digital converter in conjunction with a 10 kcps clock permits the computer to count the time between impulses and to register the times in memory by making a list of interspike intervals. Other inputs to the digital converter tell the computer which of several nerves is firing an impulse, which receptor or receptors is being illuminated, and the duration of illumination. Ultimate control of the computer and of the various stimuli to each receptor is maintained by a programmed timer (Milkman and Schoenfeld, 1966). The timer is programmed in advance of the experiment with a sequence of runs containing the desired periods of illumination of the various receptors. Normally the sequence will alternate between "experimental" and "control" runs in which the experimental runs involve inhibition and the controls do not. The data from each run is collected by the computer and stored in memory as a series of interspike intervals. For future reference a permanent record of the data is made on digital magnetic tape.

In addition to collecting and preserving data the computer monitors each run by plotting the reciprocal interspike interval versus time for each receptor and by typing the number of intervals per coarse time mark for each receptor. A glance at the plotter and typewriter outputs tells the experimenter about the various parameters of the experiment including the state of adaptation of the eye, the response of the receptors to inhibition, and the quality of optical isolation by the fiber optics system. Normally, these two monitors are the only data processing attempted during an experiment. At the completion of an experiment data may be read back into the computer for further processing. For the experiments in this thesis the processing normally involved the calculation of inhibitory coefficients.

Measuring The Inhibitory Coefficient

The inhibition between two ommatidia is described in Chapter I by equations (1) and (2) that define the inhibitory coefficients in terms of the excitation, response and inhibitory threshold of both ommatidia. As stated in Chapter I, this thesis neglects transient inhibitory phenomena and deals only with steady-state inhibition. In Chapter III some experiments are described that map the inhibitory fields in *Limulus* eye by measuring the strength of the inhibition that spreads across the eye from a cluster of four ommatidia - the reason for using four ommatidia as a source of inhibition is explained in the next chapter. The strength of inhibition is measured by the inhibitory coefficient, K, and to insure steady-state conditions K is determined by the following procedure:

(1) Taking into account the dependence of K upon the uninhibited firing rate, e_0 (Appendix I) the intensity of illumination on the test ommatidium is adjusted to give a steady-state e of about 26 impulses/sec. According to Appendix I the value of K is maximum at 26 impulses/sec.

(2) For a control run - a run without inhibition - the test ommatidium is illuminated by a single fiber with constant light intensity for 12 seconds. Typical responses of the test ommatidium during control and experimental runs are shown diagrammatically in Figure 12 (the control run is at the top). The following procedure is clarified by reference to this figure.

(3) Seventy-five seconds later an experimental run - a run with inhibition - is made. Again the test ommatidium is illuminated for 12 seconds. However, from the 6th second to the 12th second a nearby cluster of four ommatidia is illuminated through a fiber bundle. The intensity of illumination on the cluster is adjusted to give a steady-state firing rate of about 10 impulses/sec. Normally the response of only one of the four ommatidia in the cluster is recorded with the assumption that all four units respond alike.

(4) For each test ommatidium steps (2) and (3) are repeated three times at 75 second intervals with the intensity of light on the cluster increased each time. The amount of inhibition exerted by the cluster on a test ommatidium will vary with each level of light intensity giving the necessary data for the calculation of K.

(5) K is the slope of the line relating the decrease in the firing rate, e-r, of the test ommatidium to the concurrent firing rate of the cluster (see figure 4 in Chapter I). For each of the four levels of inhibition on the test ommatidium the steady-state value of e-r is obtained from the last three seconds of the six-second period of inhibition by subtracting the experimental run from the control run. The three-second period of "steady-state" inhibition is indicated in figure 12 by shading. To avoid the variable transient excitatory effects at the beginning of each run only the last 9 seconds of the 12-second experimental and control runs are subtracted.

(6) Occasionally the response of an ommatidium drifts over a period of time. That is, consecutive control runs do not contain the same total number of impulses. Normally the drift is small and fairly constant and can be avoided in the evaluation of e-r by fitting the experimental and control runs in the 3 to 6 second interval so that the difference between them in that interval is zero. Subsequent subtraction of the two runs gives a value for e-r that is relatively unaffected by drift in the preparation.

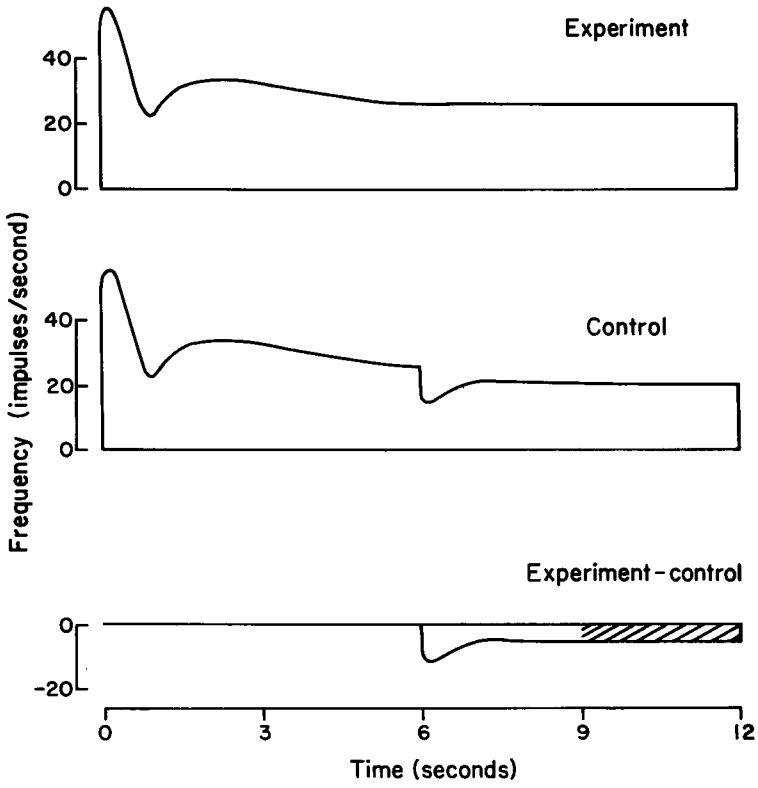


Figure 12. A diagrammatic representation of the response of an ommatidium. The upper response represents a twelve-second period of constant illumination called the "control" run. The next response is decreased by inhibition between the 6th and 12th seconds and is called the "experimental" run. Subtracting the two runs (last figure) gives the decrease in the frequency of discharge caused by inhibition. The crossed-hatched region represents the interval of "steady-state" inhibitory conditions. Refer to text for a more detailed description.

CHAPTER III

INHIBITORY FIELDS

The goal of the experiments in this thesis is to measure precisely the lateral spread of inhibition by ommatidia in the Limulus eye. With the fiber optic illumination system - described in the preceding chapter - this goal seems well within reach.

Introduction

The results of previous experiments by Ratliff and Hartline (1959) indicate that there is a certain amount of variability in the lateral spread of inhibition across the receptor mosaic. In particular, they noticed that there are "holes" in the inhibitory field of a particular ommatidium. That is, the strength of inhibition exerted by an ommatidium on one of its near neighbors may be less than that exerted on another neighbor at the same or even greater distance from it. These holes were found by Ratliff and Hartline to be more or less randomly distributed throughout the ommatidial inhibitory fields. However, disregarding the holes, the general law relating inhibition to distance (Hartline, Wagner and Ratliff, 1956) is that an ommatidium inhibits most effectively its nearest neighbors; the effectiveness diminishes with increasing distance.

It was hoped that these observations could be refined by measuring precisely the inhibitory field of a single ommatidium. In the first attempts to do so it was found that there is indeed a frequent occurrence of inhibitory "holes". Curiously, it was also found that there is a definite tendency for the holes to group around the ommatidium whose field is being measured. If this tendency were upheld by future experiments, then it would indicate that there is a depression of the inhibitory effect exerted by an ommatidium on its near neighbors and thereby would violate the generally accepted rule of a diminution of the inhibitory influence with distance. To investigate further this phenomenon a series of experiments were carried out to measure

more precisely the lateral spread of inhibition from an ommatidium.

However, it was found that to map accurately and in detail the inhibitory field of an ommatidium is extremely difficult, mainly because the strength of inhibition exerted by it is usually too weak to measure easily. Weak inhibitory influences are obscured by the variability in the response frequency, and therefore, to determine the magnitude of the influence of one ommatidium on another requires averaging of many repeated runs both with and without inhibition (refer to Chapter II). The length of time required to collect enough data for the calculation of one inhibitory coefficient severely limits the number of measurements that can be made, that is the number of ommatidia that can be mapped, in a single experiment.

Hartline and Ratliff (1958) have shown that the inhibitory influences exerted on any ommatidium by other ommatidia always combine by simple addition. In other words, the strength of inhibition exerted by a cluster of ommatidia on its neighbors is greater than that exerted by any member of the cluster. Therefore, an alternative to mapping the inhibitory field of a single ommatidium is to map the field of a cluster of ommatidia. Hopefully, the inhibitory field of a cluster will be proportional to that of a single ommatidium. There is no way as yet to determine if this will be the case; however, the chances that it will be are increased if the ommatidia within the cluster act together as though they were "one". To do this the cluster should be small enough to appear to the neighboring ommatidia as a point source of inhibition and, at the same time, be large enough to exert measurable amounts of inhibition. These criteria seem to be satisfied by a cluster of four ommatidia.

A Mapping Experiment

The results of the initial attempts to map an ommatidial inhibitory field indicate that the strength of inhibition exerted by an ommatidium was too weak for its field to be measured with precision, whereas the inhibition from a small cluster of four ommatidia seemed adequate. Consequently, the revised technique for mapping an inhibitory field is to illuminate a cluster of four ommatidia with the fiber optic bundle that is described in Chapter II, and then measure the strength of inhibition exerted by the cluster on the surrounding ommatidia.

The scale drawing in figure 13 of the lateral eye illustrates for one particular experiment, the location of the cluster and of the nearby ommatidia that are used to map its field - the dorsal direction on the eye is down and the anterior direction is to the left. The large circle below the intersection of the dotted lines indicates the location of the fiber bundle covering four ommatidia. Each of the smaller circles represents the facets of one ommatidium and the circles containing x's represent the facets of ommatidia whose nerve fibers are placed on wick electrodes behind the eye. To determine the strength of inhibition exerted by one ommatidium on another it is necessary to record the response of both. As mentioned in the preceding chapter the response of only one of the four ommatidia in the cluster is recorded with the assumption that all four units respond alike. This assumption seems reasonable since each ommatidium in the cluster receives essentially the same light intensity from the fiber optic bundle, and since in general it was found that equal light intensities evoked approximately equal firing rates from a number of ommatidia in a given eye. The preparation of the eye for most mapping experiments including the one described in figure 13 follows the procedure outlined in Chapter II plus the dissection described below.

The optic nerve is dissected with fine glass needles into several small bundles. One of the bundles is placed on an electrode and the eye is searched with a probe light to locate the position in the

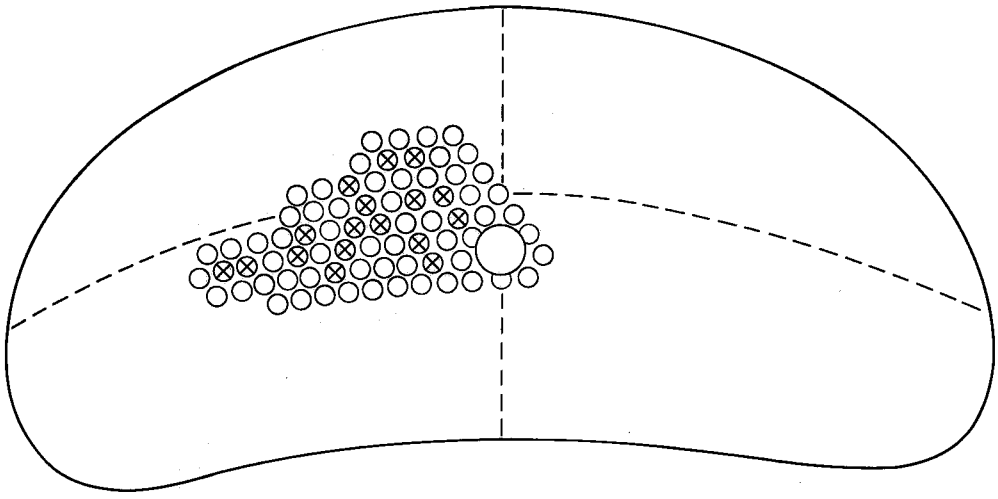
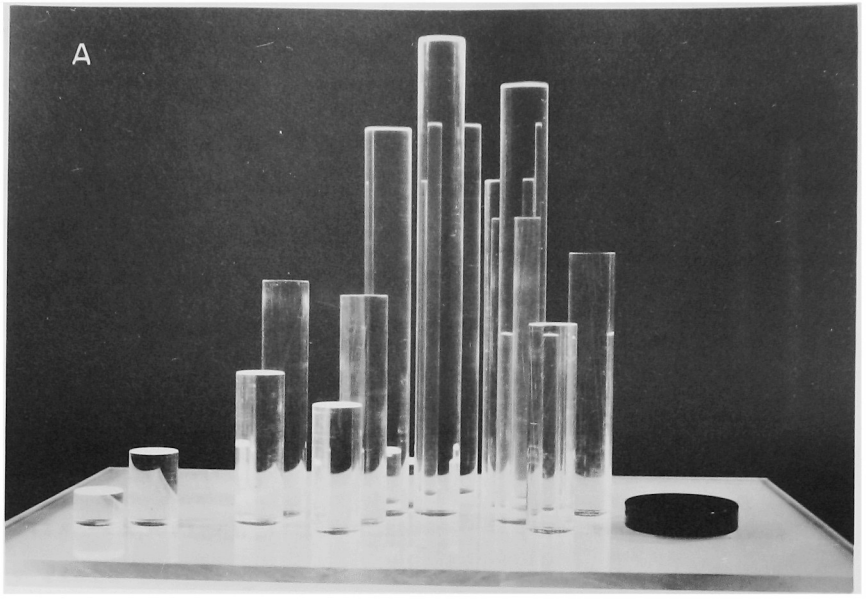


Figure 13. A scale drawing of the lateral eye illustrating the arrangement of ommatidia in the "mapping field" for a particular experiment described in the text. The solid line denotes the outline of the eye (15 mm x 7 mm); the dorsal direction is down and the anterior direction is to the left. The dashed lines - used as coordinates - divide the eye into equal sections; the antero-posterior line roughly follows the curvature of the cornea. Each of the small circles represents an ommatidial facet. The circles containing x's represent the facets of ommatidia in the "mapping field", that is the ommatidia whose nerve fibers are placed on recording electrodes behind the eye. The large circle below the intersection of the dashed lines indicates the location of the fiber bundle.

retinal mosaic of the ommatidia represented by the nerve fibers in the bundle. Each bundle of nerve fibers is tested until one is found that represents a group of ommatidia near the center of the eye. Ommatidia in the periphery of the eye are avoided in mapping experiments because their optic axes diverge 30^{o} to 40^{o} from the normal to the corneal surface (Waterman, 1954) making optical isolation difficult if not impossible. On the other hand, the optic axes of ommatidia nearer the center of the eye are approximately normal to the surface, and using the technique described in the last chapter optical isolation of these ommatidia is assured. Once located, the appropriate bundle of nerve fibers is placed on a wick electrode. The ommatidia whose nerve fibers are in the bundle constitute a "mapping field" represented in figure 13 by the circles containing x's. The next and often most difficult step in the dissection is to locate in one of the remaining nerve bundles, a fiber from an ommatidium lying on the periphery of the mapping field. When found, the nerve fiber is placed on a second wick electrode. Normally this requires the testing of many individual nerve fibers until the appropriate one is found. Once located, it is placed on a second wick electrode. The ommatidium represented by the nerve fiber becomes one of the cluster of four - indicated by the large circle in figure 13 - that is illuminated by the fiber optic bundle for a source of inhibition.

To repeat, the objective of the experiment in figure 13 is to measure the strength of inhibition exerted by the cluster on the ommatidia in the mapping field. This was done by measuring an inhibitory coefficient for each ommatidium in the field using the methods outlined in the preceding chapter. The results are shown in figure 14 by a three-dimensional lucite model. The black disc represents the location on the eye of the fiber optic bundle illuminating the cluster. Each lucite rod corresponds to an ommatidium and is located in the model as the ommatidia are located on the eye. The height of each rod is proportional to the value of the inhibitory

Figure 14. A three-dimensional lucite model illustrating the magnitude of the inhibitory effect exerted by a cluster of four ommatidia at various distances from the cluster. Figures A and B are different views of the same model. The black disc represents the location on the eye of the fiber optic bundle, 500 μ in diameter, illuminating the cluster. The transparent lucite rods correspond to the ommatidia in the "mapping field" and are located in the model according to the arrangement in Figure 13. The height of each rod is proportional to the inhibitory coefficient. Figure A is a ventral view of the model with the anterior direction to the left; Figure B is a dorsal view.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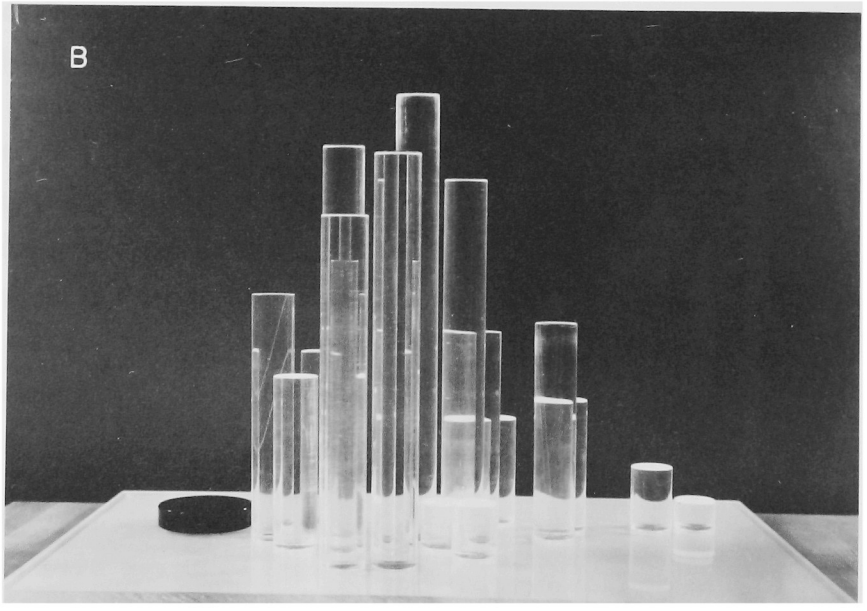

B

coefficient. It is apparent from the model that the inhibitory coefficient has a finite value near the source of inhibiton and increases to a maximum at some distance from the source. After reaching a maximum value, the inhibitory coefficient decreases monotonically to zero at a distance far from the source. The maximum is located 1.3 millimeters or 5 ommatidial diameters from the source of inhibition and the zero point is approximately 3.3 millimeters or 13 ommatidial diameters from the source. Figure 14a is a ventral view of the model with the anterior direction to the left.

The decreased inhibitory effect of the cluster on nearby ommatidia with respect to more distant ommatidia supports the results of the initial experiments that show a tendency for "holes" in the inhibitory field to group around an ommatidium (see the preceding section). Further inspection of the results in figure 14a suggests that the term "holes" - used by Ratliff, Hartline, and Lange (1965) to describe ommatidia that receive relatively little or no inhibition from neighboring ommatidia - should not be used to describe the general depression of the inhibitory coefficient near the center of the field. With increasing distance from the source of inhibition it is apparent that the value of the inhibitory coefficient increases "somewhat" uniformly and not abruptly as it would if the center of the field where surrounded by "holes". For this reason, the complex spatial distribution of the inhibitory coefficient is probably not caused by the concentration of "holes" about the center of the field, but instead may be due to some effect that changes more gradually with retinal separation.

Configuration of the Inhibitory Field

In the experiment that was just described the inhibitory coefficients were measured for ommatidia located in the anterior direction from the source of inhibition. In other experiments measurements in the posterior, dorsal and ventral directions indicate that the spread of inhibition across the eye is not symmetric. The asymmetry is shown in figure 15. The normalized inhibitory coefficient is plotted

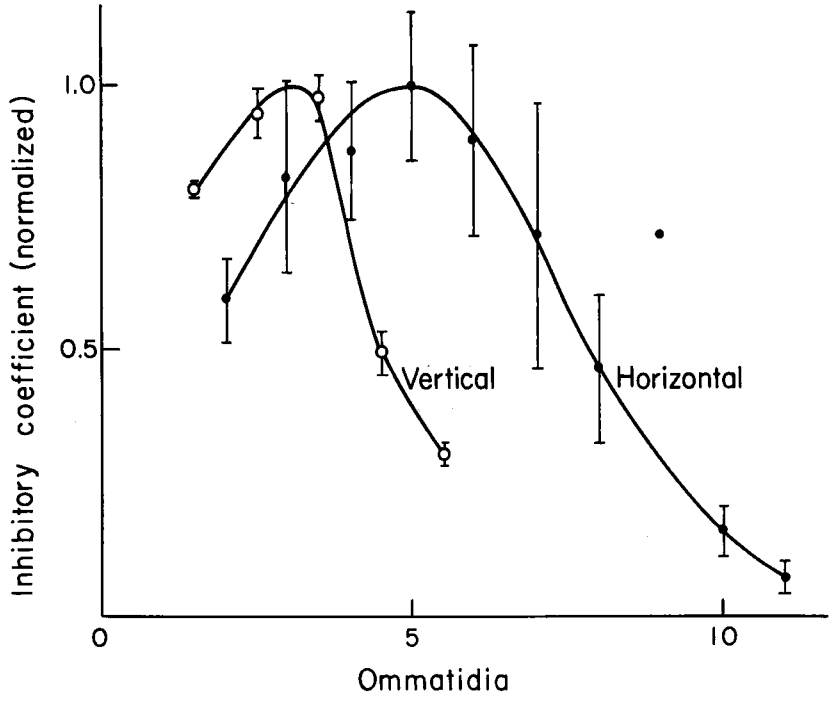


Figure 15. The dependence of the magnitude of the inhibitory effect on the separation of ommatidia in the retinal mosaic. The magnitude of the effect (measured by the "normalized" inhibitory coefficient) is plotted on the ordinate as the function of the distance from the source of inhibition in ommatidial diameters on the abscissa. The coefficients measured in the dorsal and ventral directions from the source of inhibition are nearly identical and are plotted together on the "vertical" curve; the same is true for the antero-posterior or "horizontal" direction. Each point on the vertical curve is the average of three experiments. Each point on the horizontal curve is the average of four to five experiments with one exception: the point above the horizontal represents the only measurement made at the ninth position in the antero-posterior direction. The spread of the data is indicated by the vertical bars. The data are normalized by assigning the maximum inhibitory coefficient in each experiment a value of one and adjusting the other coefficients proportionately. For theoretical considerations the two curves can be approximated by Gaussian functions: the vertical curve with a function having a peak value of 0.06 which decreases by 1.25 standard deviation units at 2 ommatidial diameters on either side of the peak; the horizontal curve with a function having the same peak value of 0.06 which decreases by 1.5 standard deviations units at 4 ommatidial diameters.

on the ordinate as the function of the distance from the source of inhibition in ommatidial diameters on the abscissa. The measurements in the dorsal direction are nearly identical to those in the ventral direction and the results of both are plotted together on the curve labeled "vertical". Each circle on the vertical curve is the average of three experiments. Similarly, the measurements in the anterior and posterior directions are nearly identical and the results are plotted together on the "horizontal" curve. Each point on the horizontal curve is the average of four to five experiments. The one exception is the point above the word horizontal which represents the only inhibitory coefficient measured at the ninth position in the anterio-posterior direction.

The data presented in figure 15 were normalized by assigning the maximum inhibitory coefficient in each experiment a value of one and adjusting the other coefficients proportionately. Some theoretical considerations in this chapter and in Chapter IV require an estimate of the strength of inhibition exerted by one ommatidium. To obtain such an estimate it is recalled that the original data were obtained by mapping the spread of inhibition from a cluster of four ommatidia. Dividing the original inhibitory coefficients by four, it is found that for all of the vertical and horizontal mapping experiments the average value of the maximum inhibitory coefficient is 0.06 ± 0.02 which agrees fairly well with the value of 0.1 published by Hartline and Ratliff.

The variation of the inhibitory coefficient in the vertical direction as compared to the horizontal direction indicates that the inhibitory field is shaped like an ellipse. Using the data in figure 15 a contour map of the inhibitory field was constructed. It is shown in figure 16. The concentric, elliptically-shaped contours were drawn to scale using as a guide the experimental points on the vertical and horizontal axes. Each contour is iso-inhibitory, and together the contours measure the spread of inhibition across the eye from the shaded ommatidium in the center. The largest contour

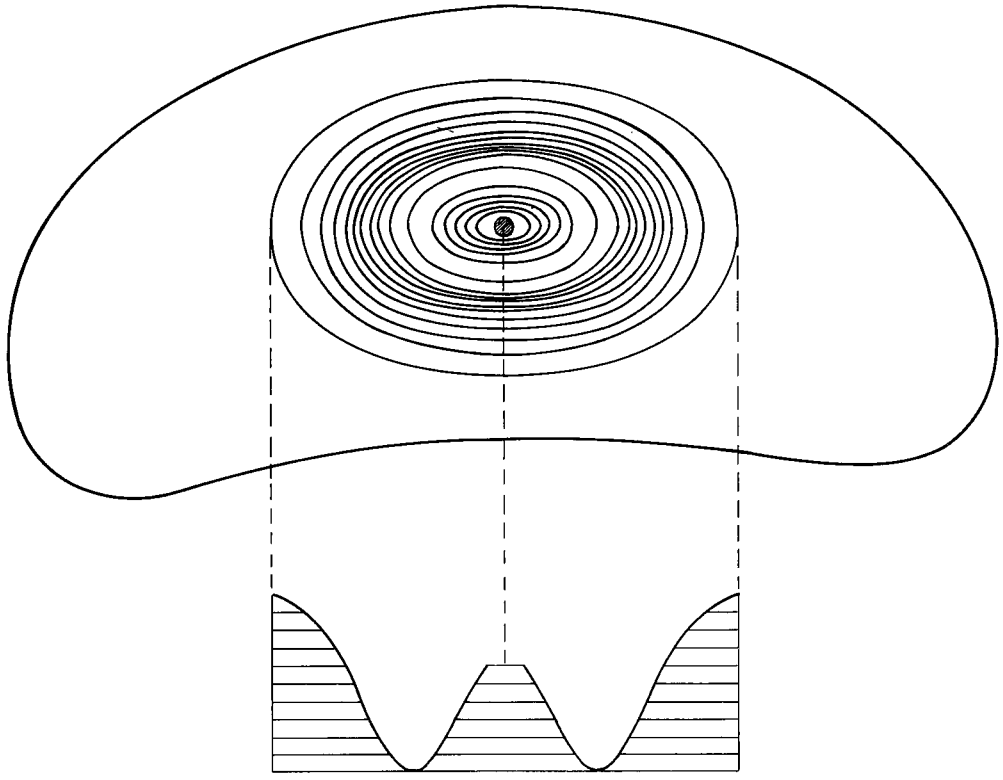


Figure 16. A contour map of the inhibitory field. The heavy solid line represents the perimeter of the eye (the dorsal direction is down and the anterior direction is to the left). Each concentric ellipse represents an iso-inhibitory contour, and together the contours measure the spread of inhibition across the eye from the shaded ommatidium in the center. The contours were drawn to scale using as a guide the points on the horizontal and vertical curves in figure 15; that is, the intercepts of the contours with the vertical and horizontal axes correspond to the data in figure 15 - the contours themselves were sketched in by hand. Data for the two contours nearest the center of the field were obtained by extrapolating the curves in figure 15. The shaded ommatidium does not exert measurable amounts of inhibition on ommatidia located outside the largest contour which represents an inhibitory coefficient of zero. A cross-section of the contour map (shown below the eye) gives the distribution of the inhibitory coefficient in the horizontal direction. The two minima indicate the location of the peak values of the inhibitory coefficient; the zero values are located at the extreme edges of the cross-section.

corresponds to an inhibitory coefficient of zero indicating that the shaded ommatidium does not exert measurable amounts of inhibition on ommatidia that are located outside of the contour. The zero points of inhibition in the horizontal and vertical directions were obtained by extending the curves in figure 15 to the abscissa. It should be emphasized that the experimental data in figure 16 are the intercepts of the contours with the vertical and horizontal axes of the eye and that the contours themselves were sketched in by the author. The asymmetric shape of the inhibitory field supports the earlier results by Hartline, Wagner, and Ratliff (1956) who, through an extensive series of experiments, determined the contours for several levels of inhibition and reported that "the inhibition diminished with increasing distance, and the diminution was more rapid in the dorso-ventral direction than in the antero-posterior". They failed to detect the initial increase of inhibition with distance because their nearest measurements were made at distances beyond the inhibitory maxima in each direction.

The number of ommatidia included within the largest contour in figure 16 is approximately 300 or about one-third of the total number of ommatidia in the eye. With the data in figure 15 it is possible to compute the total inhibitory effect of the 300 ommatidia on the central ommatidium. The computed sum of the inhibitory coefficients can then be compared to the experimental sum obtained by Lange (1965) using antidromic inhibition. To carry out the computation it is assumed that on the average the mutual inhibitory influences between two ommatidia are equal. More explicitly, the strength of the inhibitory influence - as measured by the inhibitory coefficient - exerted by the central ommatidium on the neighbor is assumed to be equal to the mutual effect by the neighbor. It is necessary to make this assumption because calculating the inhibitory effect from the surround is just the reverse operation of the mapping experiment. For the calculation each of the 300 ommatidia is assigned an inhibitory coefficient according to its location on the contour map.

The curves in figure 15 were extrapolated to obtain inhibitory coefficients for the nearest neighbors of the shaded ommatidium in figure 16. Hartline and Ratliff (1958) showed that the inhibitory effects on a given ommatidium from surrounding neighbors sum linearly. The sum of the 300 inhibitory coefficients is 7. Lange (1965) measured values as large as 2. The discrepancy between the two values is due in part to an omission of inhibitory "holes" in the calculated value. Unfortunately, too little is known concerning the frequency of occurrence of holes in the field of a single ommatidium to include them in the calculation.

The total inhibition on any ommatidium as measured by the sum of the inhibitory coefficients, $\sum_{\substack{j=1 \\ j \neq p}}^{n} K_{pj}$, may be large enough - whether it be 2 or 7 - to cause some pecularities in the mathematical behavior of the system of simultaneous equations (2) that describes the inhibitory interactions of n ommatidia. In particular, the system of equations (2) does not possess a unique solution for an arbitrary set of e_p's, K_{pj}'s, and r^o_{pj}'s. This means in physiological terms that a given pattern of illumination on the eye could evoke one of several possible response patterns, depending on irrelevant circumstances. In a detailed mathematical study Melzak (1962) proved that the system of equations (2) has a unique solution for every set of e_p's and r^o_{pj}'s if and only if $\sum_{\substack{j=1 \\ j \neq p}}^{n} K_{pj}K_{jp} < 1$ for all values of p up to and including n.

This criterion could be applied to the contour map in figure 16 by setting n equal to 300 - the approximate number of ommatidia in an inhibitory field - and by assuming that the lateral spread of inhibition from each of the 300 ommatidia is given by the field in figure 16. To arrive at the sum of the product of the inhibitory coefficients would indeed be tedious. Moreover, the result would be an overestimate due to the lack of consideration of holes in the field. It was found, however, that the result depends primarily on the maximum value of K_{pj} and on the number of ommatidia, n, which in

this case equals 0.06 and 300 respectively. For these values of K_{pj} and n Melzak (personal communication) reports that the inequality is satisfied with "plenty of room to spare". Therefore, the multiplicity of solutions to the system of equations (2) has no physiological correlate in the Limulus eye; it operates in the range where solutions are unique.

Earlier studies on the functional organization of sensory systems defined a receptive field in terms of the excitatory influences exerted on a receptor by its surround (Hartline, 1938 and 1940). Later the definition of a receptive field was extended to include any effect on the response of a receptor from its surround (Kuffler, 1953). According to this broader definition, the receptive field - or in the case of the Limulus eye, the inhibitory field - is defined by the spatial distribution of the inhibitory coefficient. Since an inhibitory effect is a depression of activity, then it is appropriate to represent the inhibitory field by negatives of the inhibitory coefficients. This is how the inhibitory field in figure 16 is to be interpreted as indicated by the horizontal cross-section located below the eye. The depressions in the cross-section indicate the relative decrease in the frequency of response of ommatidia on the anterio-posterior axis due to inhibition exerted by the shaded ommatidium in the center. Maximum inhibitory effects are represented by the minima in the curves. (Note that the cross-section turned upside down is identical to the horizontal curve in figure 15.)

Referring again to the contour map, notice that the elliptically-shaped inhibitory field resembles to some extent the oblong shape of the eye. This resemblance becomes all the more interesting when one considers the projection of the optic nerve on the retinal mosaic (see Appendix III). Briefly, it is found that nerve fibers from subunits of the optic nerve project to ommatidia that are associated in horizontal strips on the eye. The striped projection of the optic nerve on the eye corresponds to the long axis of the asymmetric inhibitory field, making it possible for subunits of the optic nerve

to send information to common or nearby points in the central ganglion from ommatidia that inhibit one another over considerable distances. Refer to Appendix III for a more detailed discussion.

The presentation of the data in figure 16 can be carried one step further by using the methods of cartography for constructing three-dimensional maps (Jenks and Brown, 1966). The surface in figure 17 is a three-dimensional map of the inhibitory field in parallel perspective which gives the illusion of depth. The construction contains no more information than the contour map in figure 16, and is included only as an aid for visualizing the general configuration of the inhibitory field. The hole in figure 17 corresponds to the shaded ommatidium in figure 16 and the curvature of the lines immediately surrounding the hole is based on data extrapolated from the experimental curves in figure 15. This is necessary because the technique used to map the inhibitory field cannot measure coefficients for ommatidia closer than $1\frac{1}{2}$ to 2 diameters from the center of the source of inhibition.

To summarize briefly the last three sections, a fiber optics illumination system was used to measure the lateral spread of inhibition exerted by ommatidia in the Limulus eye. It was found, however, that the inhibitory field of a single ommatidium cound not be determined with precision due to the inherent problems associated with the measurement of small signals. A compromise solution to the problem was to map the inhibitory field of a cluster of four ommatidia. The results are striking and inpart unexpected. The inhibitory field as measured by the spatial function of the inhibitory coefficients shows a uniform depression near the center of the field with the peak of the function appearing at some distance from the center. The function defines a large, elliptically-shaped field with its major axis in the antero-posterior direction on the eye. The field contains approximately 300 ommatidia; however, less than one-third of that number receives the bulk (75%) of the inhibitory effects exerted by a small cluster in the center. The configuration of the inhibitory

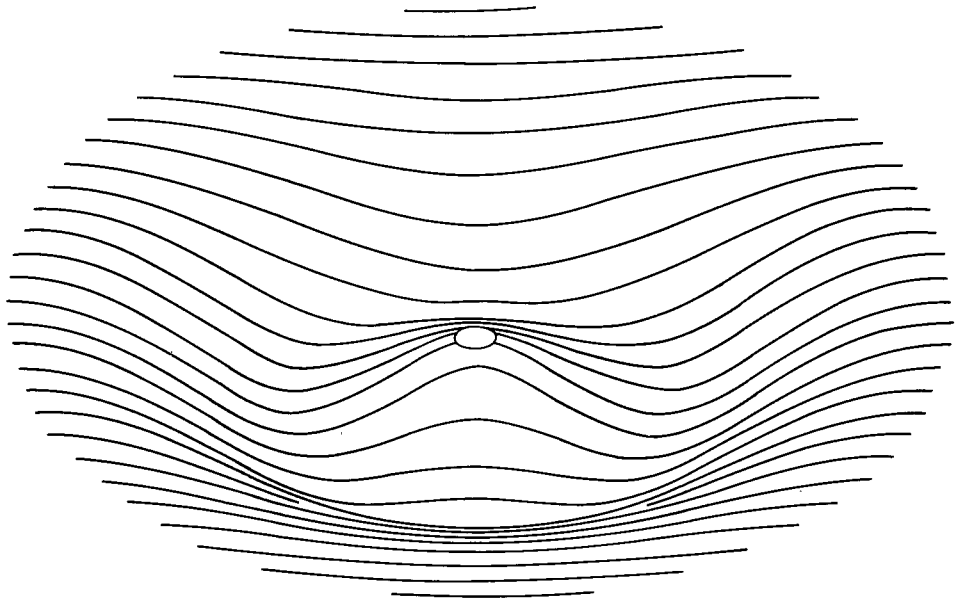


Figure 17. A three-dimensional map of the inhibitory field in parallel perspective. The map was constructed from the concentric ellipses in figure 16 using the methods of cartography outlined in Jenks and Brown (1966). The major axis (antero-posterior) of the inhibitory field lies horizontal. The open circle corresponds to the shaded ommatidium in figure 16.

field was found to be similar for a number of experiments in a sizable part of the eye.

Inhibitory Thresholds

The experiment by Ratliff and Hartline in figure 5 indicates that the inhibitory threshold is inversely related to the inhibitory coefficient. The results of the mapping experiments were expected to support this observation. Unfortunately, the data are not consistent. For example, in several mapping experiments the inhibitory threshold and the inhibitory coefficient are inversely related, in other experiments they are directly related, and still in other experiments there seems to be no relationship at all. It is apparent that nothing can be said from these observations about a correspondence between them. However, there are reasons for believing that the coefficient and threshold are related, and the fact that a particular relationship was not observed in the mapping experiments is probably due to systemmatic errors in calculating the inhibitory effects.

Suppose that all of the points in the upper graph in figure 4 were systematically shifted vertically by one impulse. As a result of the shift the intercept on the abscissa (inhibitory threshold) would decrease to approximately one-third its original value, whereas the slope (inhibitory coefficient) would remain the same. It is found that such a "vertical" shift can be introduced by the procedure that was used to calculate the inhibitory coefficient.

As described in Chapter II an inhibitory coefficient is measured by the decreases in the response frequency of an ommatidium under various levels of inhibitory input. The decrease in frequency is obtained by subtracting an experimental run - a run with inhibition - from a control run - a run without inhibition (refer to figure 12). However, as pointed out in Chapter II, the response level of the ommatidium may drift slightly between runs. To compensate for this drift the experimental run is slid along the frequency axis (ordinate)

until its response - in an interval immediately preceding the onset of inhibition - matches that of the control. This method will adequately compensate for the drift in the response level if the response characteristics, that is the waveform of firing (see figure 12), of the ommatidium being tested does not change from run to run.

However, the response characteristics of receptors generally do change to some degree during an experiment probably as a result of some unknown adapting processes. Fortunately, the changes are approximately constant from run to run, thereby introducing a systematic error that for any given ommatidium is constant for each calculation, that is for each level of inhibition. Consequently, the points that determine the inhibitory coefficient (see figure 4) are shifted equally along the ordinate affecting the x-intercept (inhibitory threshold) much more than the slope (inhibitory coefficient). The degree of shift varies from receptor to receptor resulting in a variation in the threshold that could obscure any relationship between it and the inhibitory coefficient.

Interpretation of the Inhibitory Field

The striking feature of the inhibitory field in the Limulus eye is that the inhibition exerted between neighboring ommatidia is weaker than that exerted between somewhat more widely separated ommatidia. The mutual inhibitory effects are maximal for ommatidia separated by 3 to 5 diameters, depending on their relative location on the eye, and decrease to zero for ommatidia separated by more than 8 to 13 diameters (see figure 15).

Suppose for the moment that the configuration of the inhibitory field is the result of competing excitatory and inhibitory effects arranged so that the inhibitory field is the algebraic sum of the two effects. For example, limiting the discussion to the horizontal direction on the eye, let the excitatory and inhibitory effects be represented by two exponential functions, an excitatory function that falls to a negligible value at five ommatidial diameters and an

inhibitory function that falls to a negligible value at 13 diameters. The parameters of the two exponential functions can be adjusted so that their algebraic sum is equivalent to the "horizontal" curve in figure 15. Since the "horizontal" curve can be represented in this way by the combination of excitatory and inhibitory effects, let us carry the supposition one step further to see if there are any candidates for an excitatory effect that could compete with a monotonic decreasing inhibitory effect to produce the inhibitory field. There are at least two possibilities. One is an excitatory effect due to scattered light and the other is an effect caused by the decremental conduction of excitation over fine nerve branches of the lateral plexus. Both of these possibilities should be considered.

A) Scattered Light

In mapping an inhibitory field it is conceivable that some of the measured inhibitory coefficients are smaller than the actual coefficients due to the added excitation of scattered light. If light scatter exists at all, its maximum excitatory effect would be felt in the region surrounding the source of inhibition - the same region where the measured inhibitory effects depressed. However, using the techniques described in Chapter II, the degree of optical isolation of an ommatidium is sufficient to decrease the intensity of scattered light below the impulse threshold of the surrounding ommatidia. To exert a detectable excitatory effect on the firing rate of an ommatidium the subthreshold scattered light must act in concert with light from another source. In a mapping experiment the other source is the single fiber which is used to illuminate each ommatidium for which an inhibitory coefficient is measured. The possibility of error introduced by scattered light can be tested by purposely introducing the equivalent of scattered light and measuring its effect on the firing rate of an ommatidium. Actually this amounts to measuring the increment threshold of an ommatidium with background intensities that are equal to those used in mapping experiments - intensities that cause an ommatidium to fire approximately 25

impulses/sec (see Chapter II). The experiment is performed as follows:

(1) An ommatidium is optically isolated with a single fiber and its frequency of response is recorded by a wick electrode. The intensity of light, I, on the ommatidium is adjusted to give a firing rate of less than 25 impulses/sec. If subthreshold excitatory effects cannot be detected at frequencies below 25 impulses/sec - in this case 18 impulses/sec - then they certainly will not be noticed at frequencies higher than that value.

(2) The ommatidium is illuminated with a sequence of 5 runs separated by 75 second-intervals. In the first run the ommatidium is illuminated for 10 seconds with intensity I, in the second run for 10 seconds with a much lower intensity ΔI, and in the third run with the combined intensities, $I + \Delta I$. To control the state of adaptation of the ommatidium the fourth run is a repeat of the second, and the fifth is a repeat of the first.

(3) With I held constant step (2) was repeated many times using a different ΔI each time.

(4) To insure steady state conditions the response frequency of the ommatidium was averaged over the last 5 seconds of the 10-second period of illumination.

The results for two ommatidia from different eyes are given in figure 18 which plots the increase in frequency caused by adding ΔI to I on the ordinate versus the frequency due to ΔI alone on the abscissa. In one experiment (open circles) the background intensity I alone caused the ommatidium to fire 18.6 impulses/sec and in the other experiment (filled circles) the ommatidium fired 17.4 impulses/sec. The results of the two experiments are nearly identical and therefore the following discussion will consider them together with an average firing rate of 18 impulses/sec. The reason for measuring the increment threshold sensitivity of an ommatidium to low light intensity (ΔI) superimposed on a steady background (I) is that ΔI mimics the effect of scattered light. In other words, I represents the normal - or in this case slightly below normal - intensity that is used to excite an ommatidium when its inhibitory coefficient is measured in a mapping experiment. The small increments ΔI represent various levels of scattered light. If it can be shown that any level of ΔI, which alone does not cause an ommatidium to fire impulses, will

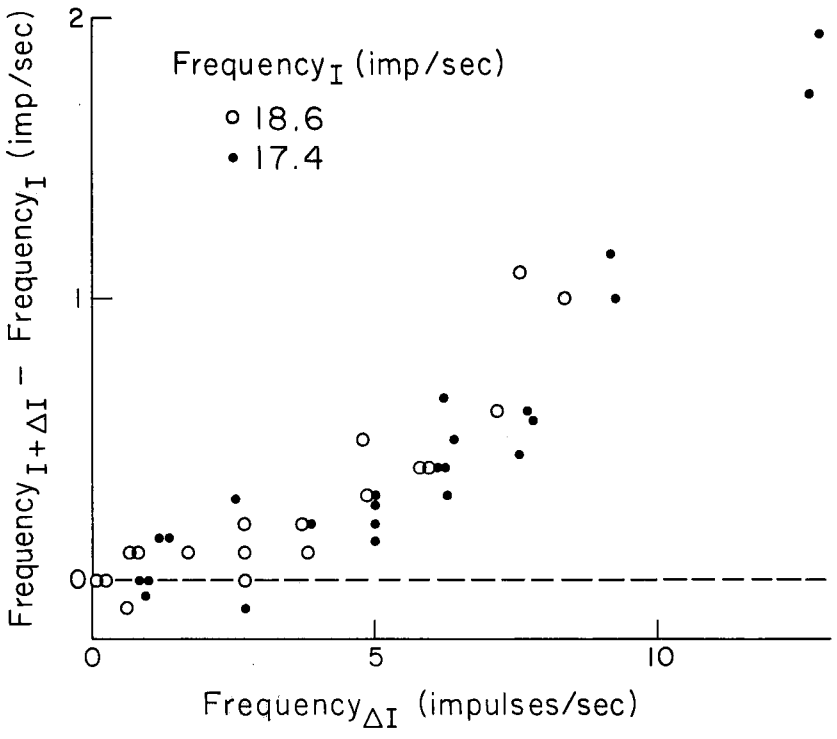


Figure 18. The steady-state response of an ommatidium to small increments in the incident light intensity. The data from two separate experiments on different ommatidia are plotted together. The steady "background" intensity, I, evoked a discharge of 18.6 impulses/sec from one of the ommatidia (open circles) and 17.4 impulses/sec from the other (dots). The increase in the response of either ommatidium to a small increment, Δ I, of the background intensity, I is plotted on the ordinate as a function of the response to Δ I alone on the abscissa. The transient effects resulting from the increments in light intensity are neglected - only steady-state responses are considered. See text for a detailed description of the experiment.

not increment the firing rate when superimposed on a much brighter intensity I, then it can be safely concluded that "subthreshold" scattered light can have no excitatory effect in a typical mapping experiment. Indeed this is the case. Note in figure 18 that at the point on the abscissa where the frequency evoked by ΔI becomes zero the increment in frequency caused by adding ΔI to I (the ordinate) is also zero. Therefore scattered light which is too weak to initiate impulses on its own, will not increase the firing rate of an ommatidium that is already firing 18 impulses/sec. It is not necessary in this argument to consider "superthreshold" scattered light because its excitatory effects can be immediately detected in a mapping experiments (see the discussion on optical isolation in Chapter II).

A family of curves has been plotted for various background intensities. For I's that evoke firing rates below 18 impulses/sec, it was found that the curves are shifted to the left of the one in figure 18 whereas higher intensities shift the curves to the right. When the curves which are shifted to the left begin to intercept the ordinate at $\Delta I = 0$, then "subthreshold" scattered light is causing a detectable effect. This effect however is not noticed until the background intensities are low enough to cause an ommatidium to fire less than 10 impulses/sec. In every mapping experiment the ommatidia - whose coefficients are being measured - are made to fire approximately 25 impulses/sec which is high enough to eliminate the effects of "subthreshold" scattered light. Therefore, it is difficult to see how the complex configuration of the inhibitory field could be an artifact of the measuring technique.

B) Local Neural Excitation

Eliminating scattered light as a source of excitation, the other possibility worth considering is an effect caused by the decremental conduction of excitation over fine nerve branches in the lateral plexus. There has never been any reason to suspect that the lateral plexus mediates excitatory as well as inhibitory effects. However,

if excitatory effects exist, then it should be possible to separate them from the inhibitory effects by selectively abolishing the inhibition with ethanol (MacNichol and Benolken, 1956).

> Several experiments were performed following a procedure similar to the one for mapping experiments (this chapter). The nerve fiber from an ommatidium adjacent to the source of inhibition was placed on a wick electrode and the ommatidium was optically isolated with the single fiber optic instrument. The source of inhibition was a nearby cluster of four ommatidia which was illuminated by a fiber bundle. The inhibition exerted by the cluster on the adjacent ommatidium was measured and then abolished with a 4% solution of ethanol in sea water. The ethanol solution was introduced by a mechanical syringe through a small hole (300 μ) drilled in the cornea with a 5/0 dental burr approximately 600 μ from both the cluster and the single ommatidium. In each experiment the injection of one microliter (1×10^{-6} liters) of the ethanol solution abolished the inhibition within one minute. Illumination of the cluster after the ethanol injection did not perturb the steady firing rate of the adjacent ommatidium. Inhibition returned at full strength with the injection of several microliters of sea water.

It was found that no excitatory interactions could be detected among neighboring ommatidia when lateral inhibition was blocked with ethanol.

Presumably, the mechanism of action of ethanol in the Limulus eye is to selectively block the synapses that mediate inhibition. A similar observation was made by Bernhard and Skoglund (1941) who found that ethanol abolished the "off" response in the vertebrate retinal ganglion cell. On the other hand, it is known that ethanol also can effect the excitatory processes in nerve membranes. Using the voltage clamp technique, Moore, Ulbright, and Takata (1964) studied the effects of ethanol on the squid giant axon. They found that ethanol reduced the nerve membrane conductance for both sodium and potassium ions, thus depressing the excitability of the axon.

In the experiments on the Limulus eye described above it was found that the injection of excessive amounts of ethanol, that is five to ten times the amount necessary to abolish inhibition, would

depress the firing rate reversibly. However, doses of ethanol that were just sufficient to abolish inhibition did not decrease the overall rate. On the contrary, several experiments indicated a slight increase in the "uninhibited" firing rate following the injection. It should be emphasized that this effect was not caused by the illumination of neighboring ommatidia, and therefore it cannot be interpreted as a local spread of excitation. The effect has not been thoroughly investigated; but if it is confirmed by future experiments, then the increase in the "uninhibited" firing rate may be interpreted as a release from self-inhibition (Stevens, 1964) indicating that lateral and self-inhibition have a common physiological mechanism.

C) Conclusion

Based on the results of A) and B) it may be concluded that the complex configuration of the inhibitory field cannot be readily explained either by scattered light or a local neural excitation. Apparently the configuration of the inhibitory field is not caused by competing inhibitory and excitatory effects. One interpretation of the particular configuration may be an arrangement of the inhibitory interconnections in the lateral plexus in which the number of connections between ommatidia increases with distance, reaches a maximum, and then decreases to zero. In other words the curves plotted in figure 15 may represent distribution functions that measure the number of inhibitory interconnections on the ordinate versus the distance between ommatidia on the abscissa. However, with the histological techniques that are presently available it has not yet been possible to determine the origin, branching, and termination of the nerve fibers in the plexus.

Receptive Fields: Limulus vs. Vertebrate

How does the inhibitory field in the Limulus eye compare with the receptive fields in other retinas? Unfortunately, there have been no reports of studies on the receptive fields in retinas as "simple" in organization as the Limulus eye. On the other hand, an extensive amount of work has been done on the receptive fields of ganglion cells in the more "complex" vertebrate retina, dating back to Hartline's original experiments (1938 and 1940) on the frog, alligator and other cold-blooded vertebrates. It may seem fruitless to compare two retinas of such divergent complexities as those of the Limulus and vertebrate; however, as pointed out in the following discussion, there are several significant similarities between them as well as some important differences.

It is well-known that the vertebrate central nervous system receives visual information via the many thousands of nerve fibers that emanate from an equal number of retinal ganglion cells. Briefly, the ganglion cell is a third-order neuron whose dendritic structure branches laterally to cover an area containing thousands of receptors. Presumably, the response of a ganglion cell can be influenced by any of the large number of receptors with which it has anatomical connections. Hartline (1938 and 1940) defined the receptive field of a ganglion cell as the area on the retina within which stimulation causes it to discharge. The definition was modified by Kuffler (1953) to include all areas that can influence the response of the ganglion cell, whether these be excitatory or inhibitory influences.

The present technique for measuring the receptive fields in a vertebrate retina is to record the response of a ganglion cell with a microelectrode (cf Granit, 1947 and Kuffler, 1953) while exploring the region surrounding the cell with a small spot of light. With few exceptions the receptive fields are found to be divided spatially into concentric and antagonistic regions, an excitatory center with an inhibitory surround and visa versa. The excitatory and inhibitory influences appear to converge on a ganglion cell presumably through anatomical connections on its dentritic structure.

In the Limulus retina, on the other hand, the inhibitory influences from an ommatidium diverge to surrounding ommatidia via axon collaterals in the nerve plexus. The mechanism of interaction in the vertebrate retina seems to be the opposite of that in the Limulus retina. This is not so. The apparent difference between the receptive fields is the result of the different methods that were used to measure them and not an indication of fundamentally different interacting mechanisms.

Ratliff (1965) has shown mathematically that either receptive field may be represented as converging or a diverging system and that the two representations for any particular system are mathematically equivalent. With the proper techniques it would be possible to show experimentally that the two fields are equivalent. For example, suppose that the response of several ganglion cells could be recorded while a small spot of light illuminated a nearby area on the retina. To be sure, the technical problems are prohibitive - or the experiment would have been done by now - nevertheless, the method is equivalent to the one described in this chapter for mapping the inhibitory fields in the Limulus eye. Conversely, suppose that in the Limulus eye the response of just one ommatidium is recorded while the surrounding retinal mosaic is explored with a small spot of light. This technique, in effect, measures the inhibitory influences converging on a single ommatidium, and thereby mimics the experiments on the vertebrate retina by Hartline, Kuffler, and others. In addition this technique is somewhat similar to the one used by Hartline, Wagner and Ratliff (1956) to measure in the Limulus eye several iso-inhibitory contours which were found to be similar in shape to those in figure 16. Therefore the receptive fields in both retinas are similar to the extent that each one can be treated as either convergent or divergent depending on the experimental (or theoretical) circumstances.

The Limulus and vertebrate receptive fields, however, differ in one very important respect: the vertebrate receptive field is typically a combination of excitatory and inhibitory influences,

whereas the interaction in the Limulus eye, as far as it is known, is purely inhibitory. For this reason a comparison of the two fields should be conducted on the common property of the inhibitory influences, that is, the Limulus inhibitory field should be compared to the inhibitory part of the vertebrate receptive field.

Separation of the latter into its excitatory and inhibitory parts is difficult because usually both influences respond to the same stimuli. However, Wagner, MacNichol, and Wolbarsht (1963) have found that in the eye of the common goldfish the excitatory and inhibitory influences on a ganglion cell may be chromatically separated. Using a small spot of light of the appropriate color, they were able to "dissect" the receptive field into its opponent parts. These receptive fields did not have the common center-surround configuration, but instead contained an overlapping of the excitatory and inhibitory systems. The light sensitivities of the two systems were maximal in the center of the field, but diminished toward the periphery at different rates so that the influence of one predominated in the center and that of the other in the surround.

Although the predominate central influence does not have a counterpart in the Limulus retina, the surround influence - of the inhibitory type - resembles the Limulus inhibitory field in several important respects. The inhibitory effects in both cases are exerted throughout the field, and more importantly the effects are graded with the distance from the field center. This statement must be qualified by adding that the inhibitory effect in the Limulus eye first reaches a maximum before tapering off toward the periphery, whereas the comparable effect in the goldfish eye decreases monotonically from the center of the field.

The opponent-color arrangement of the receptive fields of some ganglion cells in the goldfish retina bears a striking resemblance to the configuration of the fields of certain cells in the lateral geniculate of the rhesus monkey (Wiesel and Hubel, 1966). The retinal receptive field of these cells is concentrically arranged

into an excitatory or inhibitory center with an opponent surround, the center and surround having different spectral sensitivities. From their results, Wiesel and Hubel concluded that the opponent effects do not overlap with different spatial distributions as in the goldfish, but have the usual center-surround arrangement as seen in the retinas of other vertebrates. However, their measurements of the spatial distributions of the opponent effects are not detailed enough to compare with the configuration of the Limulus inhibitory field.

Rodieck and Stone (1965) investigated the possibility of interpreting the response of cat retinal ganglion cells to moving stimuli in terms of the known properties of the receptive field. In his quantitative formulation of the ganglion cell response Rodieck (1965) arbitrarily chose to represent the receptive field with the sum of two Gaussian functions, a narrow positive one for excitation and a wider negative one for inhibition. The sum of two Gaussians adequately describes the symmetric, triphasic shape of the receptive field, but as Rodieck points out other functions will do as well. Apparently, two Gaussian functions with similar characteristics also could be used to describe the overlapping excitatory and inhibitory components in the goldfish receptive field. In fact, most of the detailed analyses on the vertebrate retina indicate that the two influences spread across the field with diminishing effectiveness that could readily be described by Gaussian functions. However, the spatial distribution of either component in the vertebrate receptive field - as far as it is known - is not comparable to the complex configuration of the inhibitory field in the Limulus eye.

It is interesting to compare the relative size of the receptive fields in the Limulus and vertebrate retinas. It was pointed out earlier in this chapter that the inhibitory field in the Limulus eye contains approximately 300 ommatidia. This represents about one-third of the total number of ommatidia which in the adult eye corresponds to an area of nearly 15 mm^2. Compare these dimensions to those in the

frog retina which is similar in size to the Limulus eye - 8 mm for the frog to 10 mm for the Limulus - but contains 10^3 times the number of receptors. The receptive field of a ganglion cell contains at least one-thousand receiptors and covers an area on the retina of about 1 mm^2 (Hartline, 1940; and Barlow, 1953). This area represents only 1/128 of the whole frog retina, whereas the Limulus inhibitory fields cover as much as 1/3 of the eye. Although the two retinas are similar in size it is clear that the dimensions of the receptive fields do not resemble one another in either relative or absolute terms.

There is one other comparison between the vertebrate and Limulus receptive fields that is worth considering. Recall that the Limulus inhibitory field is asymmetric. In the vertebrate retina the receptive fields are usually reported as being more or less symmetric; however, there are several reports that suggest otherwise. For example, Kuffler (1953) noted some asymmetry in the receptive fields of the cat retina that was recently confirmed by Rodieck and Stone (1965) and Spinelli (1966). Spinelli's analysis with moving stimuli detected many asymmetric fields and also some highly specialized line and edge detectors. Recording from the ganglion cells in the rabbit retina Barlow and Hill (1963) and Barlow, Hill, and Levick (1964) found that certain cells show a selective sensitivity to direction of movement which Barlow and Levick (1965) attributed to asymmetric inhibitory influences on the ganglion cells. Perhaps the asymmetry in the shape of the Limulus inhibitory field and the asymmetry in the response of the vertebrate ganglion cells have a common footing. A correlation between them may become evident when experiments with moving stimuli are performed on the Limulus eye.

A reminder: the vertebrate retinal ganglion cell is a third-order neuron, whereas the eccentric cell in each ommatidium in the Limulus eye is supposedly a second-order neuron which for all practical purposes behaves as first-order neuron. The elaborate properties that were described above for ganglion cells are just

what one would expect for higher-order neurons but not for first-order neurons. Therefore, the diverse complexities of the vertebrate and Limulus retinas should be kept in mind when comparing the receptive fields of ganglion cells to those of eccentric cells.

Discussion

It should be emphasized that the inhibitory field shown in figure 16 represents the spread of inhibition from a cluster of four ommatidia generally located near the center of eye. The theoretical analysis in the following chapter, however, assumes that the inhibitory field in figure 16 describes equally well the spread of inhibition from one ommatidium regardless of its location on the eye. This assumption may not be entirely justified, for it is possible that the characteristics of the field may be different in each case. For example, the sum of the effects exerted by the cluster may exceed the inhibitory thresholds of a number of ommatidia that otherwise would not be inhibited by any one member of the cluster. As a result, the inhibitory field of a particular ommatidium could be highly nonuniform. Apparently this is so, as indicated by the substantial number of "holes" that were found during the initial attempts to map the inhibitory field of a single ommatidium (see introduction, this chapter).

The results of the initial experiments also point out that it is indeed difficult to determine the actual number or distribution of the "holes", that is, to map the inhibitory field of a single ommatidium. It was for this reason that a cluster of four ommatidia was used as a source of inhibition. The inhibitory field of the cluster (figure 14) is found to be fairly uniform with only the occasional appearance of a "hole". Moreover, the field is further "smoothed" by averaging the data from several experiments. The result can be fitted with a continuous function (figure 15) which of course is convenient for the theoretical analysis in the next chapter. However, it should be kept in mind that the results in figure 15 give a deceptively smooth representation of the inhibitory field of a single ommatidium. Perhaps future experiments will provide a more realistic view.

CHAPTER IV

MACH BANDS

The inhibitory field in the Limulus eye was mapped, as described in the preceding chapter, by measuring at various points on the retinal mosaic the strength of inhibiton exerted by a small cluster of ommatidia. Perhaps another method can be found for studying the properties of the inhibitory field - a method that is not based on the interaction of a few ommatidia as in the mapping experiments, but rather one that would exploit the eye's integrative properties.

Introduction

Ratliff and Hartline (1959) have shown that the integrative property of reciprocal inhibition modifies the response of the Limulus eye to simple pattern stimulation. On the basis of an earlier observation (Hartline, Wagner, and Ratliff, 1956) which indicated that the inhibitory interaction between receptors decreases with increasing separation, they predicted the general form of the response pattern of the elements in the receptor mosaic to various spatial patterns of illumination. In particular, they reasoned that retinal inhibition would cause the enhancement of visual contrast at borders and at steep intensity gradients in the retinal image.

For example, consider the response of the eye to a step pattern of light intensity arranged so that one-half of the eye is illuminated more intensely than the other half with a sharp transition between them. A receptor that is located in the dimly illuminated half but near the transition will be inhibited not only by dimly illuminated neighbors but also by brightly illuminated ones. Therefore the total inhibition exerted on it will be greater than that exerted on the dimly illuminated receptors that are farther from the transition; consequently, its frequency of response will be less than theirs. Immediately on the other side of the transition a receptor will have a higher frequency of response than a receptor that is located well

within the brightly illuminated half but which receives strong inhibition from all of its immediate neighbors that are also brightly illuminated. Ratliff and Hartline concluded that "the differences in the activity of receptors on either side of the transition will be exaggerated and the discontinuity in this pattern of illumination will be accentuated in the pattern of neural response".

They proceeded to test these predictions experimentally by focusing on the Limulus eye various patterns of illumination and recording the neural response. Ideally one would record the response from a number of receptors at various positions with respect to the pattern of illumination, but practically one must instead record from only one receptor and shift the pattern of illumination so that the receptor can assume successively a number of different positions with respect to the pattern. The lower graph (curvilinear) in figure 21a which is redrawn from Ratliff and Hartline's 1959 paper represents the neural response to the pattern of illumination given by the upper graph (rectilinear). As Ratliff and Hartline predicted, the central discontinuity was accentuated with a maximum and minimum appearing in the response pattern as a result of the inhibitory interaction among neighboring receptors. In addition they found an accentuated response to the discontinuities in a pattern of illumination that contained a simple gradient of intensity.

The maximum and minimum appearing in the response of the Limulus eye to an intensity gradient have a psychophysical counterpart in human visual phenomena known as Mach bands. To the human observer the edges of the penumbra of a shadow cast by an object placed in front of an extended source appear as light and dark bands. The bands are named after their discoverer Ernst Mach (1865) who attributed them to a functional interaction of neighboring retinal elements. He hypothesized that the type of interaction was reciprocal inhibition and that its effect was carried with diminishing strength over the lateral network of neural interconnections in the retina. Mach concluded that the "purpose" of the inhibitory interaction was

to accentuate the appearance of borders and contours in the retinal image. The reader is referred to the recent book by Ratliff (1965) for a thorough and interesting discussion of Mach bands; their discovery, production, and physiological significance. For the purpose of this discussion the meaning of the term "Mach bands" will be extended to include the neural response to step patterns of illumination as well as to intensity gradients.

To reiterate, Mach believed that the enhancement of contours and borders by the human visual system was due to reciprocal inhibition that gradually diminished as it spread across the retina. Indeed, Ratliff and Hartline, starting with the experimental result of the diminution of inhibition with distance, predicted the general form of the accentuated response of the elements in Limulus eye to various spatial patterns of illumination. A sufficient condition for the production of Mach bands is, therefore, a diminution of the inhibitory interaction with increasing distance on the retina. The amplitudes of the Mach bands, that is the degree to which a border is accentuated, should depend on the strength of the inhibitory interaction. Weak retinal interactions should produce little or no contrast effect, whereas strong interactions would be expected to produce large effects. In addition, the width of the Mach bands should depend directly on the extent of the lateral spread of inhibition, and their shape should be determined by the way in which the inhibitory effects diminish with distance. In other words, one would expect that the configuration of the inhibitory field determines the various characteristics of the Mach bands: their amplitude, width, and shape.

If this is true, then it might be possible to derive the configuration of the inhibitory field from the experimentally measured Mach bands. Before such a derivation is attempted, however, there are several important points to consider. First, a mathematical model of the inhibitory system in the Limulus eye must be constructed in order to calculate the response patterns to several patterns of

"illumination" using various configurations of inhibitory fields. A comparison of the calculated response patterns to the inhibitory fields that produced them should reveal the necessary information for deriving the actual configuration of the inhibitory field from the experimental Mach bands.

However, the results from the model may indicate that characteristics of the Mach bands are not uniquely determined by the configuration of the inhibitory field. If the calculated Mach bands prove to be rather insensitive to gross changes in the inhibitory field, then the experimentally measured Mach bands will contain very little useful information concerning the actual configuration of the inhibitory field. If on the other hand the characteristics of the Mach bands are directly correlated with the inhibitory field, then the experimental and calculated Mach bands can be compared and hopefully the general form of the inhibitory field can be deduced.

The last and most important point to consider is whether or not the comparison between the theoretically calculated Mach bands and the experimentally measured ones is valid. For example, the mathematical model that is described in the following section is a very simplified representation of the Limulus eye, and for this reason the calculated Mach bands may not bear any resemblance to those measured in the eye. There may be, in addition, a number of technical problems associated with simple pattern illumination of the eye whereas none should exist for the model. The remainder of this chapter is devoted to a study of these points. Briefly, the results of the study show that the general form of the inhibitory field as deduced from the characteristics of the Mach bands agrees with the one measured in the mapping experiments; however, the characteristics of the Mach bands alone do not provide enough information to determine the detailed configuration of the inhibitory field.

Theoretical Calculations

A theoretical treatment of the inhibitory system in the Limulus eye was reported by Ratliff and Hartline in their original paper (1959) on Mach bands. At that time a mathematical model of the inhibitory system was largely speculative because the exact law relating the magnitude of inhibition to retinal separation was not known; in fact, it was the absence of such a law that stimulated their theoretical analysis. They hoped to determine the general form of the spatial function of interaction by comparing the experimentally measured Mach bands to the output of the mathematical model incorporating various forms of the spatial function - which is precisely the point of this chapter.

To do this they formulated the set of simultaneous equations (1) to describe the response of a receptor array that is exposed to a step pattern of illumination. Numerical solutions to the set of equations were obtained by an iterative method of successive approximations. Although none of the solutions were described in detail, Ratliff and Hartline (1959) made the general observation that "any hypothetical law that postulates the inhibitory coefficients decreasing and the inhibitory thresholds increasing with increasing receptor separation will predict, for appropriate intensity distributions similar to those we have used, maxima and minima in the patterns of receptor response that will be like those we have observed in actual experiments". (The effects due to the variations in the inhibitory threshold are not considered in the theoretical calculations in this section because, as noted in the last chapter, the results of the mapping experiments do not show a well-defined relationship between the inhibitory threshold and the inhibitory coefficient.)

At first, Ratliff and Hartline obtained the numerical solutions by hand after many hours of calculations. Later Ratliff (1965) programmed a digital computer to solve the system of equations, thereby decreasing substantially the time required for one calculation. Taking full advantage of the computer's capabilities, he increased

the size of the model and included a plotter output to observe the successive steps in the iterative solution. With these additions the computer completed one calculation every three hours. A significant decrease in the time for each calculation would require a much larger computer than the Control Data 160-A laboratory model that was used by Ratliff.

Fortunately, the Mathematics Group at IBM Research, Yorktown Heights, New York, took the problem under consideration. Dr. Don Quarles, a member of the group, programmed an IBM 7094 computer to solve directly the set of N simultaneous equations (2) for $N \leqslant 100$. The set of equations is arranged in the program to represent a "one-dimensional" model of the Limulus eye. The model may contain up to 100 receptors that are, in effect, strung in a row and that interact with one another according to the set of equations (2) in which the uninhibited firing rates, e_p, inhibitory coefficients, K_{pj}, and inhibitory thresholds, r^o_{pj}, are predetermined. The model is one-dimensional because the inhibitory interactions are confined to a string of receptors.

A number of solutions to the set of equations were obtained by Dr. Quarles. The values of the parameters e_p, K_{pj} and r^o_{pj} for the various solutions were determined primarily by the experimental data in the preceding chapter with some appropriate adjustments to accentuate the more subtle properties of the Mach bands. I am indebted to Dr. Quarles for the time he spent with me discussing the computed results. However, many of the results from the model will not be included in this discussion; the ones shown in figures 19 and 20 were selected especially to demonstrate the effect of the configuration of the inhibitory field on the shape of the Mach bands. The following is a detailed discussion of the values that were assigned to e_p, K_{pj}, and r^o_{pj} to obtain these results and of the prominent features of the results themselves.

The pattern of "illumination" of the model is determined by the uninhibited firing rates, e_p, that are assigned to each receptor in

the "one-dimensional" array. A simple step pattern of "illumination" was used to calculate the eight responses that appear in figures 19 and 20. This was done by setting e_p equal to 36 impulses/sec for equations 1 through 50 and to 16 impulses/sec for equations 51 through 100. The values of 36 and 16 were chosen to avoid any complications that may be caused by the nonlinearity of the inhibitory coefficient. The discussion in Appendix I points out that the value of the inhibitory coefficient depends on a receptor's uninhibited firing rate to the extent that the sensitivity of the receptor to inhibition is greatest at e_p equal to 26 impulses/sec and will decrease for e_p's above and below this value. At 16 and 36 impulses/sec the inhibitory coefficients are the same and, therefore, these firing rates were chosen as the excitation levels in the step pattern. The nonlinearity is included in the computer model so that the calculated responses would represent as closely as possible those which are measured in the eye. However, the major effects of the nonlinearity are avoided in the responses in figures 19 and 20 by the judicious choice of the uninhibited firing rates in the excitation pattern. Refer to Appendix I for a description of some "asymmetric" Mach bands that are produced by excitation patterns which do not avoid the nonlinear effects.

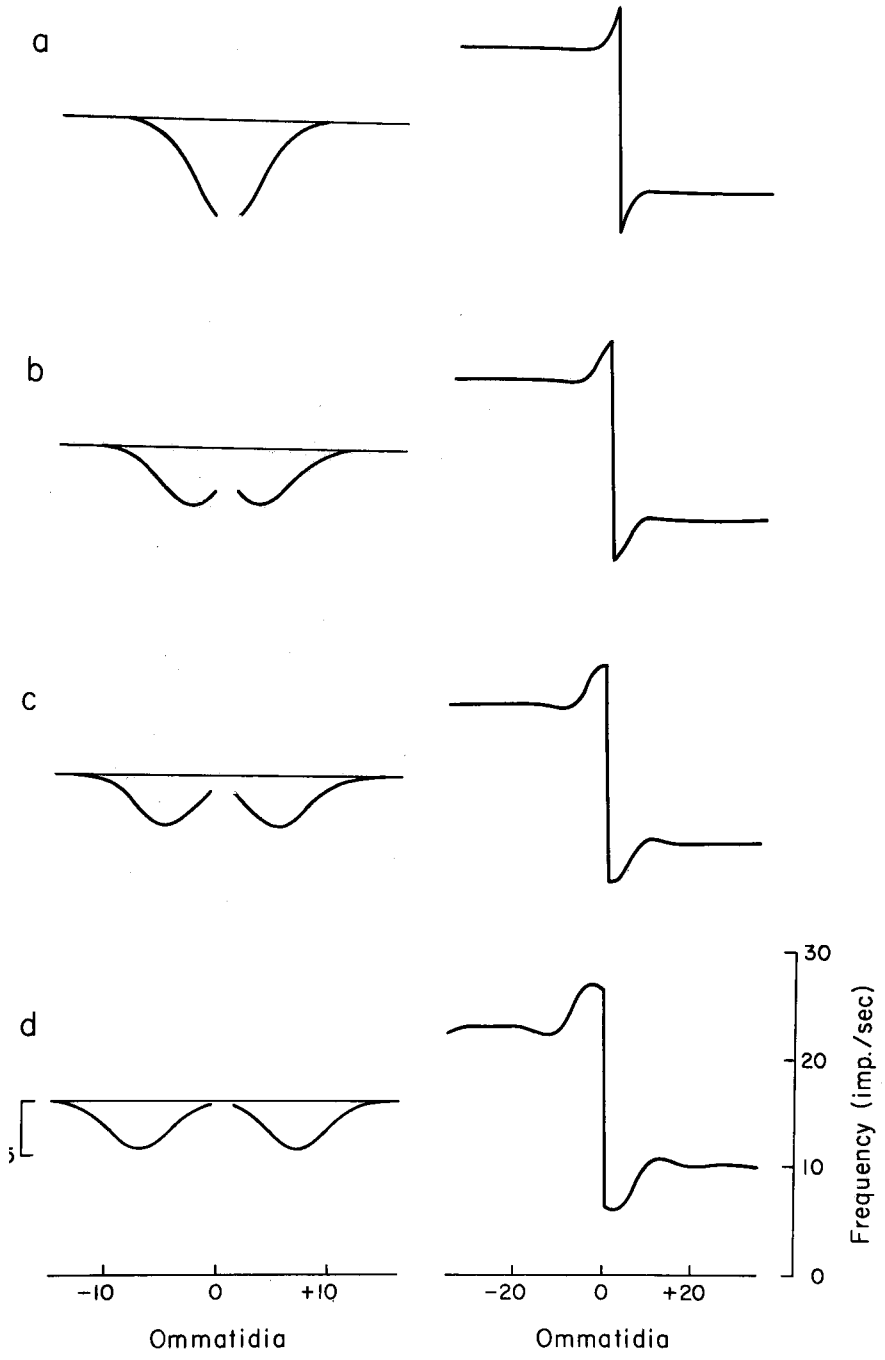
Next, a spatial function must be chosen to define the extent and magnitude of the inhibitory interactions among the model's 100 receptors. The calculated Mach bands will be compared to the experimental ones in the next section and, therefore, the "vertical" and "horizontal" components of the experimentally determined inhibitory field (shown in figure 15) are obvious choices for the spatial function. However, the "one-dimensional" computer model can treat only one function at a time, and the choice for the first set of calculations is the horizontal component - primarily because the experimental Mach bands are measured in the horizontal direction on the eye, and also because it is the more prominent of the two components. The variation of the inhibitory coefficient in the horizontal direction

is fairly well described by a Gaussian function with a peak value of 0.06 which decreases by 1.5 standard deviation units at 4 ommatidial diameters on either side of the peak. The Gaussian-shaped inhibitory field is plotted on the left in figure 19c. Note that the inhibitory field is defined by the distribution of the inhibitory coefficients whose values are plotted on the ordinate in exactly the same way as they were in figure 16. For this particular calculation each receptor in the model is assigned the "one-dimensional" inhibitory field shown on the left in figure 19c. The computer model is programmed to assign every receptor the same inhibitory field, because there is no experimental evidence to suggest otherwise.

The last values that need to be designated in order to solve the set of equations are the inhibitory thresholds. The computer model has the facility to assign to each receptor an inhibitory threshold according to an inverse relationship between it and the inhibitory coefficient. The inverse relationship was based on the experiments by Hartline and Ratliff (1958); however, as mentioned previously the results of the mapping experiments do not show a clear-cut relationship. Therefore the inhibitory threshold will be taken as zero for the calculations in figures 19 and 20. The effects that non-zero thresholds have on the calculated responses are discussed at the end of this chapter.

Once the pattern of excitation (e's) and the parameters of the inhibitory interaction (K's and r^{o}'s) are designated, then the computer can solve the set of equations for the response frequencies. The curve on the right in figure 19c plots the response frequency on the ordinate as a function of the lateral position on the model "eye" in ommatidial diameters on the abscissa. The response frequencies of only the middle 70 receptors of the 100-receptor model are plotted in figure 19c. The responses of the remaining 30 receptors contained pronounced "edge-effects" (to be described in the next section) and, for the sake of clarity, were not included. The computed response accentuates the step pattern of excitation with

Figure 19. Response patterns calculated with a "one-dimensional" computer model of the Limulus eye using various configurations of the inhibitory field. On the left are four different fields each one represented by the variation of the inhibitory coefficient (positive values downward) on the ordinate versus distance from the center of the field (in ommatidial diameters) on the abscissa. Each field is described by a Gaussian function in which the peak value of the inhibitory coefficient decreases by 1.5 standard deviation units at 4 ommatidial diameters and the total sum of the coefficients is 0.8. On the right are the four response patterns corresponding to the inhibitory fields on the left. The patterns are plotted with the computed response frequency on the ordinate and the "receptor" position in the model eye on the abscissa. In each case the pattern of excitation was a simple step function in which the uninhibited response of each receptor was 16 impulses/sec to the right of the step and 36 impulses/sec to the left. The response patterns were obtained by Dr. Don Quarles on an IBM 7094 computer. See text for details.


a
b
c
d
-10
0
+10
Ommatidia
-20
0
+20
Ommatidia
30
20
10
0
Frequency (imp./sec)

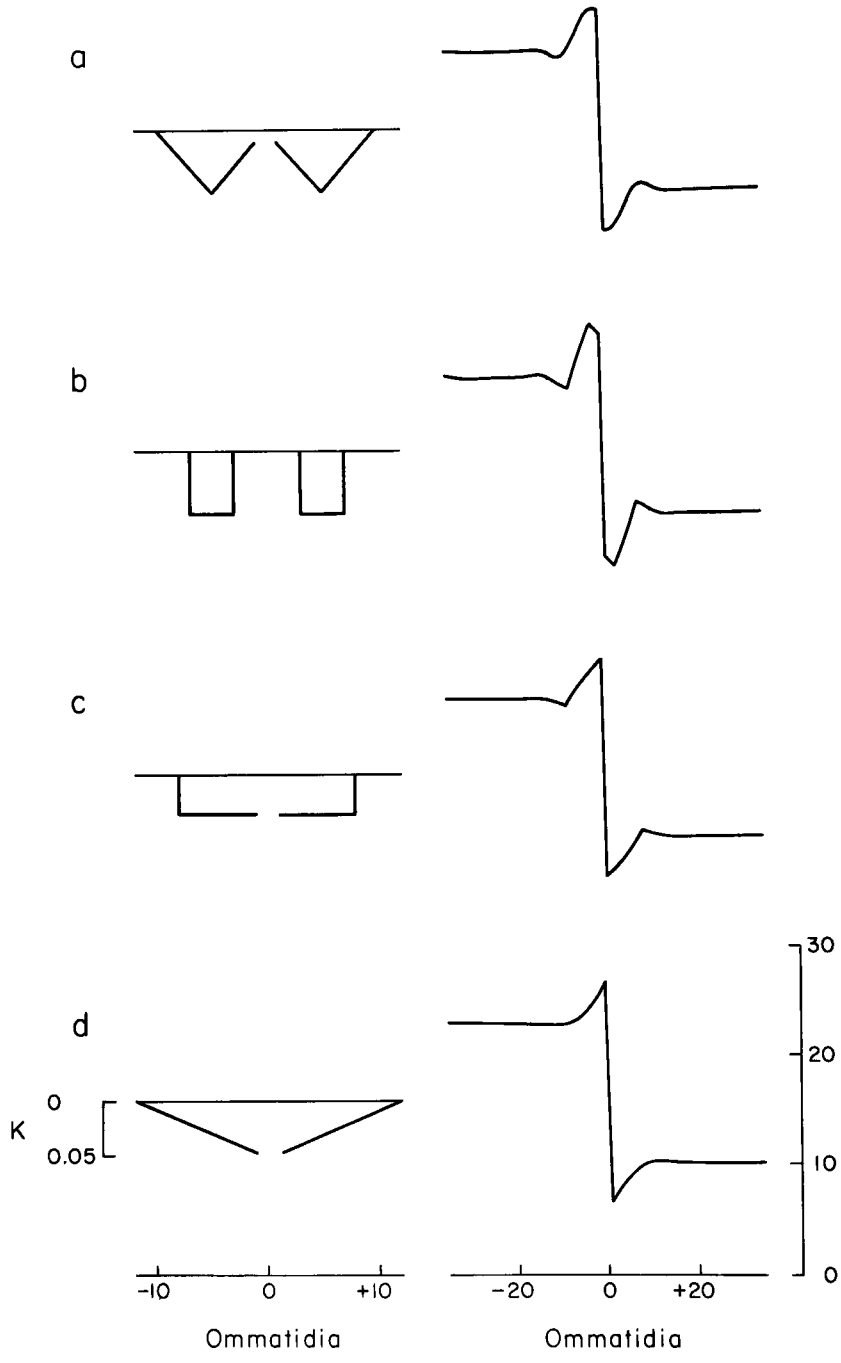


Figure 20. Response patterns calculated with a "one-dimensional" computer model of the Limulus eye using various configurations of the inhibitory fields. This figure is similar to the preceding one (figure 19) in every respect with the exception of the configuration of the inhibitory fields. These fields were chosen to emphasize particular subtleties in the response patterns. (Response patterns provided by Dr. Don Quarles.)

well-defined Mach bands that appear somewhat s-shaped and show noticeable "second-order" effects. The "second-order" effects refer to the highly damped oscillations that the Mach bands undergo while approaching steady response levels far from the discontinuity. Actually, these oscillations are "second-order" Mach bands that accentuate the primary maximum and minimum. Their appearance in the response pattern is indicative of the recurrent nature of the inhibitory interaction. To repeat, the computed Mach bands in figure 19c represent the response of the model "eye" to a step pattern of excitation when it is programmed with the horizontal component of the experimentally determined inhibitory field. It will be interesting to compare these Mach bands to those in the next section that are measured experimentally.

Having computed the Mach bands, the next step is to test their sensitivity to gross changes in the configuration of the inhibitory field. This is done by shifting the point of maximum to other positions with respect to the center of the inhibitory field. Recall that the inhibitory field in figure 19c has a maximum at 5 ommatidial diameters as determined by the mapping experiments. Shifting the inhibitory maximum to 7 diameters while preserving the same total amount of inhibition, produces different shaped Mach bands as shown in figure 19d. The characteristics of the Gaussian function have not been changed and the area under the curve has been held constant so that in each case, (a) through (d), the total amount of inhibition exerted by each receptor is the same. Notice that the Mach bands in (d) are wider and more rounded than those in (c) and that they also show a more pronounced second-order effect. On the other hand, shifting the inhibitory maximum nearer the center of the field as in (b) causes a decrease in the width of the Mach bands with a loss of the s-shaped characteristic. In (a) the inhibitory maximum coincides with the center of the field producing a marked contraction of the Mach bands with no second-order effect. To repeat, the inhibitory fields in (a), (b), (c), and (d) are described by the

same Gaussian function with only the amplitudes changed to maintain constant total inhibition.

The Mach bands in figure 19a are similar in shape to those that were measured experimentally by Kirschfeld and Reichardt (1964). Their results are shown in figure 21b. From the characteristics of the solid curve they deduced that the inhibitory field could be represented by a centered Gaussian function. Similarly, the Mach bands in figure 19a were produced by a centered Gaussian-shaped inhibitory field. A more detailed discussion of Kirschfeld and Reichardt's experiment will be given in the next section.

An analysis of the computed results in figure 19 reveals that the total width of the Mach bands is roughly equal to the width of the inhibitory field. This same result was found by Békésy (1960) who calculated the formation of Mach bands using a simple model of receptor interaction called the "neural unit". Based on his experiments on the perception threshold of two points on the surface of the skin, Békésy concluded that the stimulation of a small point on the receptor mosaic produces a local field of sensation (or excitation) surrounded by an extended refractory (or inhibitory) field. The term "neural unit" refers to the whole combination of sensation area and refractory area. Using a simplied rectangular model of the complex neural unit, Békésy calculated the transformation of stimulus patterns into patterns of sensation magnitudes - for details of the calculation see Békésy (1960). He found that discontinuities in the stimulus patterns produced Mach bands in the calculated sensation patterns and that the total width of the Mach bands is given by the width of the refractory area of the neural unit.

In order to define more accurately the shape of the neural unit Békésy (1960) measured the width of the Mach bands in the eye and on the skin of the human observer. By comparing the observed Mach bands to the appropriate calculated ones, he determined the width of the refractory (or inhibitory) area. However, Békésy found it more

difficult to determine the width of the sensation (or excitatory) area. The formation of the Mach bands, as Békésy points out, does not depend on the exact width of the sensation area as long as it is much less than the refractory area. He found, however, that the ratio of the sensation area to the refractory area could be measured by the proper selection of a stimulus distribution (see Békésy, 1960), thus enabling him to deduce the width of the sensation area. He found that the neural unit for the eye measures about 0.06 mm across its width and the one for the skin measures approximately 50 mm across. For the skin the sensation area occupies a much larger part of neural unit than does its counterpart in the eye.

To determine these features Békésy had to assume a simple model of the neural unit, thus making it impossible for him to gain any information on the detailed configuration of the excitatory and inhibitory areas of the unit. Nevertheless, Békésy's theoretical analyses of sensation patterns with the neural unit demonstrates the important one-to-one relationship between the total width of the Mach bands and the size of the refractory (or inhibitory) area. This observation, which is corroborated by the results in figure 19, should be useful in the following section for interpreting the experimentally measured Mach bands.

However, the main point for considering a theoretical treatment of the Mach bands was to find an independent method for confirming the results of the mapping experiments; therefore, the Mach bands should be analyzed in terms of the information they might contain concerning the actual configuration of the inhibitory field - not just its size. The most prominent feature of the experimentally measured inhibitory field (figure 19c) is that the point of maximum inhibition is located at some distance from the center of the field. The effect, if any, that this feature has upon the shape of the Mach bands will now be investigated by comparing the response patterns that are calculated with inhibitory fields having different configurations.

Note that the response pattern in figure 20a is similar to the one in figure 19c indicating that a linear approximation of the Gaussian-shaped inhibitory field does not alter significantly the shape of the Mach bands. If the experimentally measured Mach bands (in the following section) happen to resemble those in figures 19c and 20a, then the "real" inhibitory field probably would possess those characteristics which are common to the Gaussian-shaped inhibitory field and its linear approximation - a more precise description of the "real" field would be impossible. Evidently the fine structure of the inhibitory field cannot be determined from the shape of the Mach bands. On the other hand, the Mach bands are known to contain a certain amount of information on the size of the inhibitory field (Békésy, 1960, and this thesis), but it remains to be seen just how much information they contain on the configuration of the inhibitory field.

A simple rectangular approximation of the Gaussian-shaped inhibitory field is shown on the left in figure 20b. Note that the rectangular field retains the one very distinguishing characteristic of the Gaussian field, that is, the maximum inhibitory effects are displaced from the center of the field. The Mach bands on the right in figure 20b that are calculated using the rectangular field have a somewhat rectilinear shape that appears to be an accentuation of the s-shaped bands in figures 19c and 20a. Considering the dissimilarities between the Gaussian and the rectangular fields, it is remarkable that the calculated Mach bands bear even the slightest resemblance. Evidently, the Mach bands are very sensitive to changes in the width of the inhibitory field and much less so to changes in its configuration. This observation assumes that for any given field the total amount of inhibition, as determined by the sum of the inhibitory coefficients, is constant.

Next consider the calculation of Mach bands using an inhibitory field in which the characteristic displaced maximum inhibitory effect is eliminated. For example, the inhibitory field in figure 20c

represents a constant spread of inhibition out to a certain point, but with no effect beyond that point. Note that the resulting Mach bands are not s-shaped as in the previous response patterns; but rather they show a sharp rise (or fall) in the response frequency at the discontinuity in the excitation pattern. The Mach bands are similar in shape to those that Békésy (1960) calculated with a neural unit whose refractory (or inhibitory) area was identical to the inhibitory field in figure 20c. A similar mathematical model postulated by Taylor (1956) also treated the inhibitory area as uniform throughout. His model yielded maxima and minima in the response pattern having the same general characteristics as those shown in figure 20c.

The last type of inhibitory field to be considered is one in which the inhibitory coefficient decreases linearly from the center out to a certain point and is zero beyond that point. The "triangular-shaped" field is shown on the left in figure 20d. The calculated Mach bands in figure 20d have the same general characteristics as those in (c) of the same figure indicating that the s-shaped characteristic is lost completely when the inhibitory field does not contain a displaced maximum. Therefore, the most prominent feature of the experimental inhibitory field causes the calculated Mach bands to have a pronounced s-shape or point of inflection.

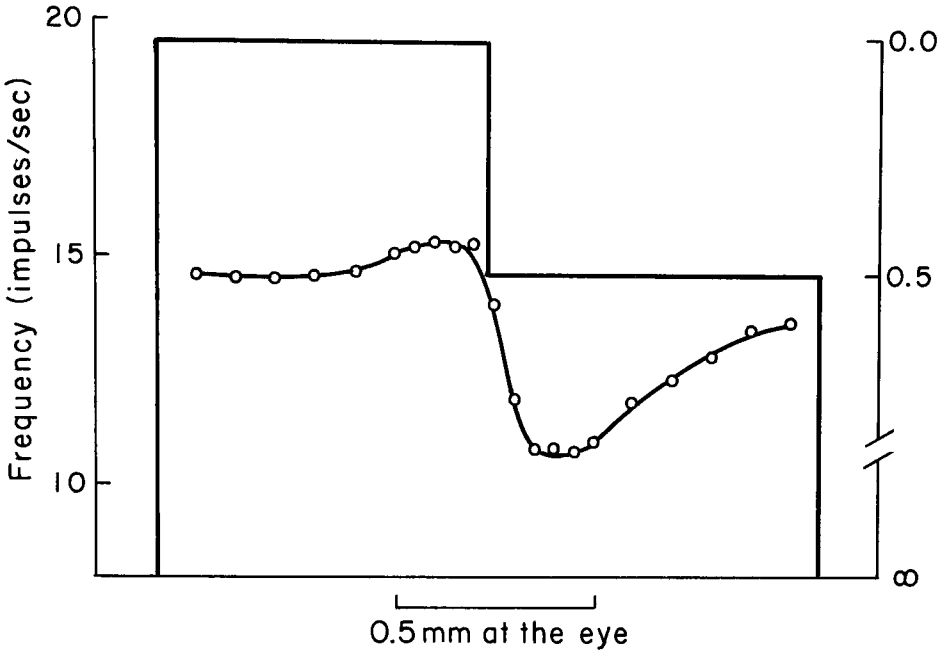
In summary, a theoretical treatment of the Mach bands, using a computer model of the Limulus eye, indicates that the width of the inhibitory field determines the width of the Mach bands, and that the configuration of the inhibitory field is expressed to some degree in the shape of the Mach bands. In particular, an inflection point in the Mach bands indicates that the maximum value of the inhibitory coefficient is located at some distance from the center of the inhibitory field; whereas, Mach bands that are shaped as a cusp indicate that the spatial function of the inhibitory coefficient probably decreases monotonically with increasing distance from the center of the field.

Experimental Measurements

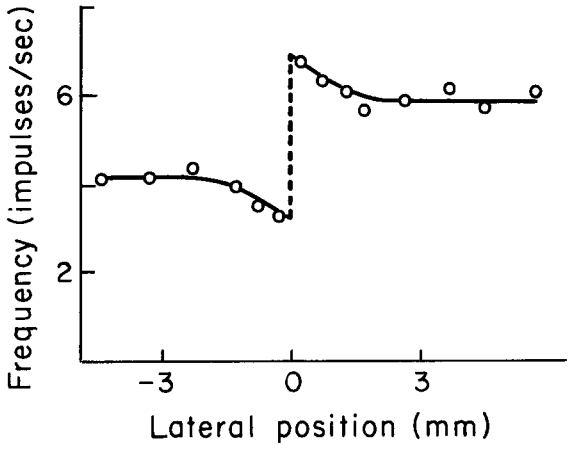
The next step is to determine which one, if any, of the calculated response patterns in figures 19 and 20 resembles the neural response pattern that is measured in the Limulus eye. However, before such a comparison can be made, it is important to ascertain that both the calculated and neural response patterns are produced by the same excitation pattern. The theoretical treatment in the preceeding section dealt with only a simple step pattern of excitation and, therefore, it is essential that the same pattern be used in the experimental measurements on the eye.

Two experiments have been reported that measure the Limulus eye response to a step pattern of illumination: one by Ratliff and Hartline (1959) and the other by Kirschfeld and Reichardt (1964). The results of both experiments are shown in figure 21. It is interesting to compare their results because each experiment employs a different technique for stimulating the eye. The technique used by Ratliff and Hartline was to focus on the eye a demagnified image of a transilluminated photographic plate. On half of the plate was blackened so that the ratio of transmitted light was 4:1. On the eye the step pattern of illumination ocvered an area (1.65 mm x 1.65 mm) containing approximately 40 receptors. According to the contour map in figure 16, approximately 300 receptors are contained within the inhibitory field of a small cluster of ommatidia. If the lateral spread of inhibition from a single ommatidium covers roughly the same number of receptors as it does for the cluster, then the pattern used by Ratliff and Hartline illuminated an area that was much smaller than the field of one ommatidium. Therefore, a substantial amount of the inhibition (approximately 80%) from each of the 40 receptors was exerted outside the area of illumination. For this reason, the Mach band effects that were produced by the step pattern probably extended beyond the illuminated area, and consequently, the curve in figure 21a probably does not represent the "complete" response to a simple step pattern. Furthermore, the illumination on the eye was zero outside

Figure 21. The discharge of impulses from a single ommatidium in response to a step pattern of illumination in various positions on the retinal mosaic. The results of two different techniques for illuminating the eye are illustrated. (A) A demagnified image of a transilluminated photographic plate (one-half blackened) was focused on the eye. The relative intensity of the pattern (rectilinear graph) is plotted on the right-hand ordinate versus the retinal position on the abscissa. The steady discharge of impulses was recorded from one ommatidium and the pattern of illumination was shifted between measurements. Each open circle on the curvilinear graph is the response frequency (left-hand ordinate) at various points of the pattern as indicated by the abscissa. Data from Ratliff and Hartline (1959). (B) Eye placed in direct contact with a transilluminated film - one-half blackened. The relative transmission of light through the film was the same as in (A) but covering a much larger part of the eye as indicated by the abscissa. As in (A) one ommatidium assumed successively a number of different positions with respect to the pattern with the response of the ommatidium given on the ordinate and the retinal position on the abscissa. Data from Kirschfeld and Reichardt (1964).


20
15
10
Frequency (impulses/sec)
0.0
0.5
∞
0.5 mm at the eye

A


6
2
Frequency (impulses/sec)
-3
0
3
Lateral position (mm)

B

the pattern (rectilinear graph in figure 21a) and each edge produced an accentuation in the response pattern, called an "edge effect", which due to the pattern's small size merged with and obscured the Mach bands occurring at the step. The continuous rise in the response frequency on the right in figure 21a indicates that the Mach band from the dimly illuminated side of the step merged with the one from the right-hand edge of the pattern. The Mach band at the edge (maximum) complemented the one at the step (minimum) and, therefore, they combined to form an s-shaped response curve. On the left hand side of the curve the step and edge produced similar Mach bands which merged to give two maxima that are separated by a slight depression. The maximum at the extreme left-hand edge is not visible because the response was measured over the central 1.5 mm of the 1.65 mm pattern. The computer model could be programmed with the triple step pattern of excitation given by the rectilinear graph in figure 21a so that the calculated responses would be appropriate to compare with Ratliff and Hartline's measurements. This has not been done mainly because the responses to simpler stimuli, such as step patterns, are thought to contain more information about the configuration of the inhibitory field.

It is apparent from Ratliff and Hartline's measurements that, Because of edge effects, the response to a simple step pattern cannot be determined by illuminating a small area on the eye. For the sake of accuracy their measurement more closely represents the response to a triple step pattern of illumination, and therefore, the curve in figure 21a should not be compared to the theoretically calculated responses in figures 19 and 20.

The other investigation of Mach bands in the Limulus eye was carried out by Kirschfeld and Reichardt (1964) who increased the area of stimulation by placing the eye in direct contact with a film whose one half had been slightly blackened. The transilluminated film covered over 90% of the eye, thereby eliminating edge effects. They used Ratliff and Hartline's technique for measuring the neural

response, that is, the discharge of impulses from only one receptor was recorded and the pattern was shifted between records so that the one receptor assumed various positions with respect to the pattern. They measured the steady-state response at each position and found that the step pattern was indeed accentuated. However, the Mach bands shown in figure 21b do not resemble in detail those obtained by Ratliff and Hartline. The discrepancy between the two measurements is due primarily to the elimination of edge effects by Kirschfeld and Reichardt's technique.

In addition, Kirschfeld and Reichardt formulated a method for deriving from the shape of the Mach bands a theoretical function that relates the inhibitory coefficients to distance. Assuming the lateral inhibitory system to be linear and the responses to be above the threshold of inhibition, they calculated that a Gaussian function with the appropriate constants would yield a good approximation to the measured Mach bands. However, the variability of the steady-state responses (see figure 21b) prevents an accurate determination of the Mach band characteristics, and therefore, the calculated Gaussian function that relates the inhibitory coefficients to distance may be in error (Reichardt, personal communication). For this reason the Mach bands in figure 21b cannot be compared to the computed responses in figures 19 and 20. It is evident from the preceding discussion that more accurate measurements of the Mach bands must be obtained, and the attempt to do so is described below.

To reduce edge effects in the measurement of Mach bands, a fiber optic instrument was designed to illuminate upon contact with the cornea approximately 80% of the eye with a simple step pattern. The construction of the instrument is described in Chapter II under the heading: Mach Band Instrument. The method for measuring the Mach bands is the same as the one used in the two preceding experiments, that is, one receptor is recorded and assumes various positions with respect to the pattern.

In order for the results from this experiment to be comparable to the computed results in figures 19 and 20 the light intensities in the step pattern must be properly adjusted to avoid any complications due to the nonlinearity of the inhibitory coefficient. According to figure 26 in Appendix I, the nonlinearity can be avoided by choosing uninhibited firing rates that are symmetric about 26 impulses/sec which corresponds to the maximum value of the inhibitory coefficient. Recall that the excitation pattern for the calculated responses corresponded to uninhibited firing rates of 36 and 16 impulses/sec. To achieve a similar excitation pattern with the fiber optic instrument a 250 μ aperture is placed directly on the eye to mask light from every receptor except the one which is being recorded. Under these conditions the receptor's response will be uninhibited. The fiber optic instrument is manuevered into position in front of the eye so that its optic axis is parallel to that of the receptor. The metallic strip that separates the two halves of the pattern is made parallel to the dorso-ventral axis of the eye, and then the instrument is lowered until it just touches the aperture which is in contact with the cornea. The light intensity of the one-half of the step pattern that happens to be directly over the receptor is adjusted to give an uninhibited firing rate of approximately 30 impulses/sec. The instrument is then moved to illuminate the receptor with the other half of the pattern whose light intensity is adjusted to give a note of approximately 16 impulses/sec. For the experiment reported in figure 22 the uninhibited firing rates were 32.8 and 18.6 impulses/sec respectively. Note that these rates are symmetric about 26 impulses/sec but that they do not correspond exactly to the excitation pattern used in the computer calculations.

The aperture is removed and the eye is illuminated with the entire step pattern for a period of 10 seconds. The nerve impulses discharged during the last 7 seconds of the period are counted. Two minutes later the instrument is shifted to a new position and the stimulus is turned on again for 10 seconds. This procedure is

repeated every 2 minutes while the pattern is shifted back and forth along the antero-posterior axis of the eye. The tabulated data from each measurement is divided by 7 to obtain an average response frequency and the results are plotted in figure 22.

The Mach bands are clearly defined. The response at the bright edge (maximum) is accentuated by 7 impulses/sec and at the dim edge (minimum) by 6 impulses/sec. A comparison of these values to the average accentuated response of 1 impulse/sec in both experiments in figure 21 demonstrates the decided advantage that the fiber optic instrument has over the other techniques for illuminating the eye with a simple step pattern. Note in figure 22 the second-order effect that appears on the left of the response maximum. A similar effect, if it exists, near the response minimum is obscured by the variability in the data. The slow increase in the response frequency on the extreme right probably is an edge effect which, due to the large area covered by the step pattern, is not continuous with the response minimum as it is in Ratliff and Hartline's result.

It is evident that the Mach bands in figure 22 are larger and more clearly defined than those in figure 21 and, more importantly, they are similar in shape to some of the computed responses in figures 19 and 20.

Comparison

In particular, the experimentally measured Mach bands in figure 22 show a striking resemblance to the computed Mach bands in figures 19c and 20a. The computed response in figure 19c was produced by a step pattern of excitation using the horizontal component of the experimentally determined inhibitory field, whereas the response in figure 20a was based on a linear approximation to the horizontal component. It is not surprising that these computed responses resemble the experimentally measured ones since the latter were produced by a step pattern of illumination that was placed in the anterio-posterior or horizontal direction on the eye. In fact, there

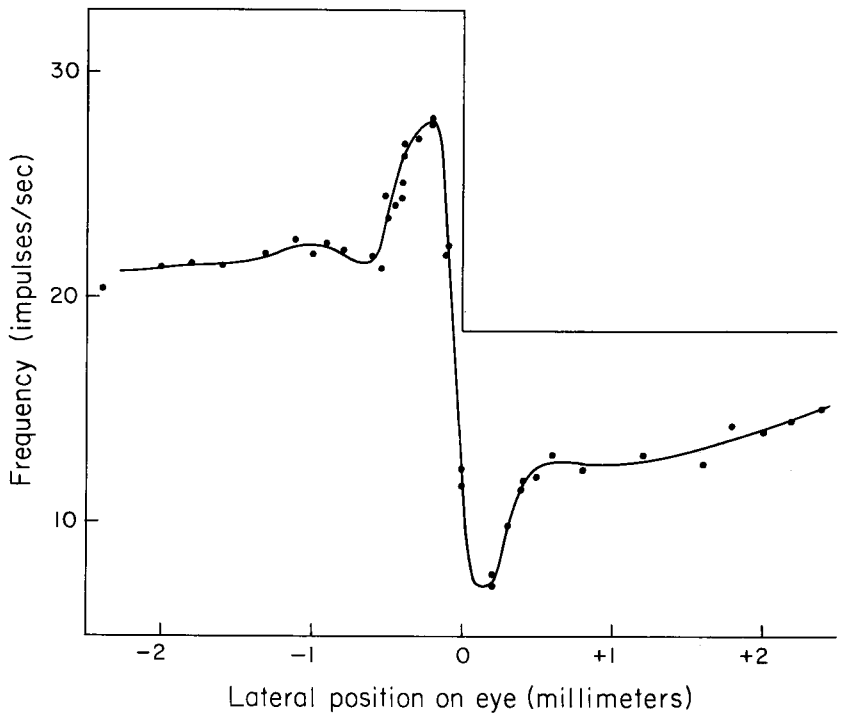


Figure 22. The discharge of impulses from a single ommatidium in response to a step pattern of illumination in various positions on the retinal mosaic. The pattern of illumination was rectangular, covering over 80% of the adult Limulus eye. It was obtained by placing a fiber optics instrument, containing over 400,000 glass fibers (see Chapter II), in contact with the eye. The intensities of both halves of the step pattern were independently adjustable. The upper (rectilinear) graph shows the excitation pattern used in this experiment. The two levels represent the rates of discharge of the ommatidium when the illumination from the fiber optics instrument was occluded from the rest of the eye by a mask with a small aperture. The lower (curvilinear) graph is the frequency of discharge from the same ommatidium when the mask was removed and the entire pattern of illumination was then placed on the eye in various positions. Each point represents a response measurement (ordinate) for the particular position of the pattern (abscissa). See text for a more detailed description of the experiment.

was every reason to expect that the Mach bands in figure 22 would be equivalent to those in figure 19c - as it turns out, although they are similar, they are not strictly equivalent. The discrepancy between them lies in the width of the bands. This point is discussed in detail at the end of this section.

It is instructive to compare in detail the experimental and theoretical Mach bands and to see, subsequently, what conclusions can be made concerning the configuration of the inhibitory field. To aid in this comparison the response pattern in figure 19c is replotted on an expanded scale in figure 23. It was pointed out previously that the computed response patterns in figure 19 undergo several distinct changes as the point of maximum inhibition is shifted away from the center of the field, one being a transition from the cusp-shaped Mach bands in (a) to the s-shaped ones in (d) and the other being a gradual increase in the second-order effect from (b) to (d). Similar changes are evident in the computed response patterns in figure 20. Notice that the experimentally measured Mach bands in figure 22 are definitely more s-shaped as opposed to being cusp-shaped. This is especially true of the band on the brightly illuminated side of the step pattern. The number of experimental points that describe the bright band (or maximum) is nearly twice that which describes the minimum and, therefore, the characteristics of the maximum rather than the minimum are more accurately represented in figure 22. The maximum also shows a pronounced second-order effect that is not evident in the minimum probably due to the paucity of data.

The salient features - s-shape and second-order effect - of the experimentally measured Mach bands strongly suggest that the spatial function of the inhibitory coefficient must have the same general characteristics as the curves in figure 15 that were determined by the mapping experiments. Based solely on the information contained in the shape of the experimental response pattern, it may be stated that the maximum value of the inhibitory coefficient does not lie in

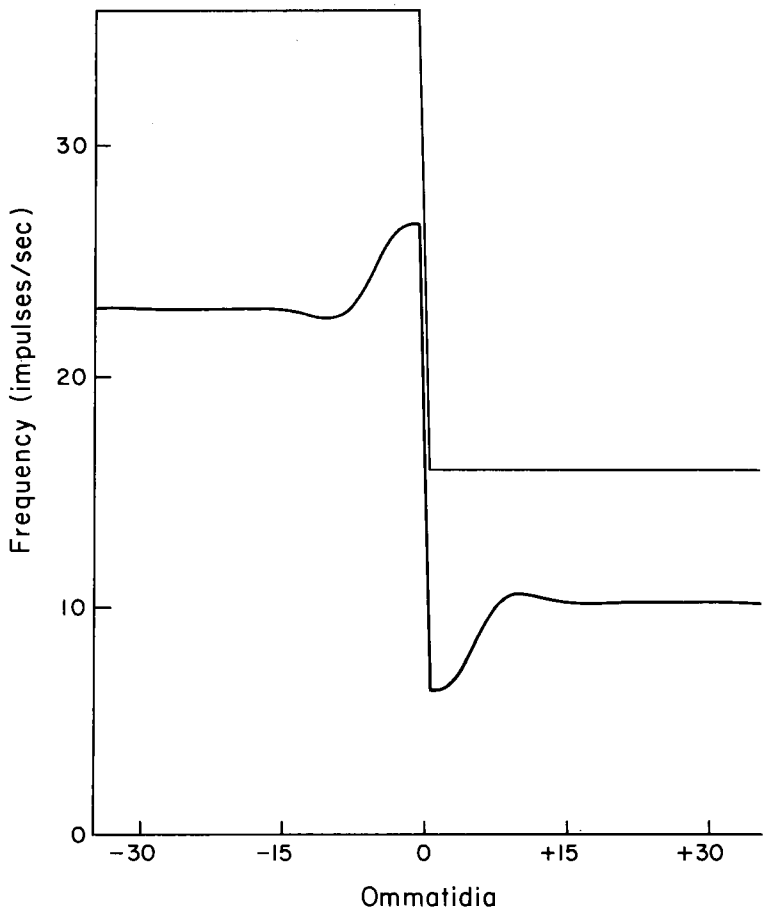


Figure 23. Computed response pattern from figure 19c replotted on an expanded scale. In addition, the pattern of excitation has been included (rectilinear graph). This pattern is of particular interest because the horizontal component of the experimentally determined inhibitory field was used in the computation. (Pattern provided by Dr. Don Quarles.)

the center of the inhibitory field but rather is located at some distance from the center. The distance cannot be determined exactly, although it is apparent from the response patterns in figure 19 that the point of maximum inhibition is separated by more than three ommatidium diameters from the center of the field. This observation agrees with the results of the mapping experiments. However, it should be clear from the above discussion that the shape of the Mach bands does not provide enough information to permit the unambiguous derivation of the detailed configuration of the inhibitory field. Also, for this reason, it should be pointed out that the theoretical method used by Kirschfeld and Reichardt (preceding section) to derive the configuration of the inhibitory field does not lead to a unique solution.

Another similarity between the response patterns in figures 22 and 23 is the amplitude of the Mach bands, that is, the size of the maxima and minima with respect to the steady response levels far from the step. The amplitude of a Mach band is determined by the strength of the inhibitory interaction and by the nature of the excitation pattern. The step-patterns of excitation in figures 22 and 23 were chosen so that the uninhibited firing rates on either side of the step would be symmetric about 26 impulses/sec, thereby producing maxima and minima that were inverted images of one another (see Appendix I). It is evident that the Mach bands in figure 23 are mirror images of one another, whereas this is not quite true of those in figure 22; however, the difference is not considered significant.

Notice that the step pattern of excitation in figure 23 is larger than the one in figure 22, but that the Mach bands in figure 23 have smaller amplitudes than those in figure 22. The amplitudes of the Mach bands should be directly proportional to the size of the step in the excitation pattern. The discrepancy can be explained only in terms of the relative strength of the inhibitory interactions in each case. For the computed response in figure 23 the strength of the inhibitory interactions among the 100 receptors can be

measured by the value of the inhibitory coefficient (0.06) which is assigned to the maximum point in the "horizontal" curve in figure 15. The value of 0.06 was obtained by averaging the maximum inhibitory coefficients from eight mapping experiments (see Chapter III).

To produce the larger amplitude Mach bands in figure 22 from a smaller step pattern of excitation, the receptors in the "real eye" must have interacted to a greater extent than those in the model eye. In the real eye the maximum value of the inhibitory coefficient - if it had been determined - probably would have been greater than 0.06. An approximate value could be obtained by comparing the experimental response pattern in figure 22 to patterns which had been computed with the same inhibitory field as figure 19c but with increasing amounts of the total amount of inhibition within the field. This has not as yet been done.

One point that has been mentioned but not discussed in detail is the relative scale on the abscissae of figures 22 and 23. Notice in figure 22 that the response frequency is plotted on the ordinate versus the lateral position on the eye in millimeters on the abscissa, whereas in figure 23 the response frequency is plotted against the number of ommatidia. The diameter of an ommatidium in an adult eye is approximately 0.25 mm, and therefore, the abscissa in figure 23 should be expanded 3.4 times in order to equate it with the one in figure 22. Plotted on equivalent scales the computed Mach bands are approximately three times wider than the experimental ones, and consequently, there may be little justification for comparing them. Nevertheless, the striking similarity between the responses in figures 22 and 23 that lead to a "tentative" confirmation of the inhibitory-field characteristics was too attractive not to be considered in detail.

The difference in the widths of the Mach bands may be due to the one-dimensional characteristic of the computer model as compared to the two-dimensional nature of the Mach band experiment. The computer model is equivalent to 100 receptors strung-in-a-row, and

therefore the inhibitory interactions that produced the response pattern in figure 23 were "confined" to the one-dimensional string of receptors. The Mach band instrument, on the other hand, illuminates a large area on the eye; thus, the response in figure 22 was produced by inhibitory influences that were propagated laterally across the two-dimensional plane of the eye. In its present form the computer model does not consider the 360^{o} spread of inhibition from each receptor, however, modifications are underway to make it do so. Hopefully, the new two-dimensional model will rectify the difference in the widths of the Mach bands, and thereby make the comparison between experiment and theory more convincing.

The difference might be resolved by comparing the computed responses in figures 19 and 20 to the results of a one-dimensional Mach band experiment. To perform a one-dimensional experiment the tip of the Mach band instrument was masked with black tape so that only a thin strip - one to two ommatidial diameters wide - of the step pattern was exposed. The masked instrument was placed in contact with the eye and an attempt was made to measure the neural response pattern. Unfortunately, the inhibitory interaction among the small number of ommatidia in the illuminated strip was too weak to measure easily and the experiment proved impractical. Recall that the first attempts to measure the inhibitory field of a single ommatidium were also impractical because of weak inhibitory effects. Clearly a number of receptors must be stimulated to produce sizable effects that are easily measured.

Perhaps the difference in the widths of the experimental and computed Mach bands is due to the lack of consideration of the inhibitory threshold in the model eye. The results of the mapping experiments showed no clear-cut relationship between the inhibitory threshold and inhibitory coefficient, and for this reason the threshold was given a value of zero for all points in the inhibitory field. However, Ratliff and Hartline (1959) found that in general the inhibitory threshold was inversely related to the inhibitory

coefficient. It was thought that the introduction of an inverse relationship may cause a "constriction" of the inhibitory field, thereby reducing the width of the Mach bands. This idea was tested by assigning to each of the 100 receptors in the model a threshold which was related to the inhibitory coefficient by the formula: $K \times r^{o} = 0.18$. Such an inverse relationship with a constant of 0.18 was found to be the best hyperbolic fit of the experimental data, although other functions would have done as well. The resulting Mach bands were similar in appearance to those in figure 23 and their width was decreased by less than 10% which hardly accounts for the large (3:1) discrepancy in width that exists between the experimental and computed Mach bands.

Summary

A theoretical analysis of the enhancement of visual contrast by the Limulus eye was carried out with the help of Dr. Quarles from IBM Research. It seemed appropriate at this time to conduct such an analysis because a function relating the magnitude of the inhibitory coefficient to retinal distance between receptors had been determined by a series of mapping experiments (see Chapter III). The main point of the analysis was to obtain some indirect evidence on the spatial function of the inhibitory coefficient that, in turn, might confirm the results of the mapping experiments.

It was found that the most prominent feature of the spatial function - a diminution in the value of the inhibitory coefficient near the center of the inhibitory field - was correlated with some distinct characteristics of the Mach bands that were produced by a model eye upon "illumination" with a simple step pattern. The characteristics are a pronounced s-shape and a slight second-order effect. The next step was to compare these characteristics to those contained in the neural response pattern of the real eye. Two teams of investigators, Ratliff and Hartline (1959) and Kirschfeld and Reichardt (1964), have measured the Mach bands that are produced in

the Limulus eye by a step pattern of illumination. However, for various reasons that are enumerated in this chapter it was felt that neither measurement should be compared to the computed responses of the model eye.

For this reason a new set of measurements were made using a fiber optics instrument to illuminate the eye. The resulting neural response pattern showed well-defined Mach bands that were s-shaped and contained second-order effects. This observation agrees with the results of the mapping experiments, but as pointed out in this chapter it cannot confirm them. The biggest stumbling-block in comparing the experimental and computed response patterns is the large discrepancy between the widths of the Mach bands. At this time there is no explanation for the computed Mach bands being three times wider than the experimental ones. Whether the discrepancy is due to the one-dimensional characteristic of the computer model as opposed to the two-dimensional nature of the Mach band experiment remains to be seen.

Two-dimensional Model: Preliminary Results

The steady-state inhibitory interactions in a two-dimensional array of ommatidia can be described by a set of simultaneous linear equations. The solution of this set of equations for various receptive-field configurations and excitation patterns is being studied by Dr. Don Quarles. Using the same straight forward matrix methods that were employed to solve the one-dimensional case, Dr. Quarles obtained some preliminary results for receptor arrays of ten by ten ommatidia and smaller - the size of the array being limited by the size of the memory in the IBM 7094 computer.

As it was pointed out in the Experimental Measurements section of this chapter, the response to a small step pattern of excitation is obscured by edge effects. To overcome this difficulty the dimensions of the receptor array must be increased to several times the width of the ommatidial receptive field. By using a fast

Fourier transform method (Cooley and Tukey, 1965) for solving the N x N set of equations, Dr. Quarles was able to conserve computer memory and thus increase the size of the two-dimensional model (the method conserves computer time as well as computer memory). With this method the size of the receptor array for the two-dimensional model can be increased to 32 x 32 on the 7094 computer.

Only a few preliminary solutions have been obtained thus far. These computations were made using a simple step pattern of excitation and a simplified circular inhibitory field in which the inhibitory coefficients were constant throughout. The computed response patterns show well-defined Mach bands containing "second-order" effects (see Theoretical Calculations, this Chapter, for the definition of "second-order" effects). In the one-dimensional calculations it was shown that the width of the Mach bands - as defined by the location of the second-order effect - is equal to the width of the inhibitory field. This correlation appears to hold also for the two-dimensional results; however, the correlation depends strongly on the total amount of inhibition exerted by each receptor. This effect together with "holes" in the inhibitory field and inhibitory thresholds must be studied in the two-dimensional model before any definite conclusions can be drawn.

Recall that the primary reason for investigating the two-dimensional model was to rectify the discrepancy between the widths of the experimental Mach bands and those calculated with the one-dimensional model. Unfortunately, the two-dimensional model has not been studied in sufficient detail - at the time of this writing - to provide an answer to this problem.

APPENDIX I

THE DEPENDENCE OF THE INHIBITORY COEFFICIENT ON THE LEVEL OF EXCITATION

The inhibitory interaction of two ommatidia is described by a pair of linear equations (1) that defines the inhibitory coefficients, K_{AB} and K_{BA}, in terms of the excitation, response and inhibitory threshold of both ommatidia. The value of the coefficient describing the action of A on B (figure 4 - upper graph) is determined by the slope of the line relating the decrease in the frequency of discharge from B to the concurrent response of A - visa versa for the action of B on A (lower graph). In the first comprehensive paper on inhibition in the Limulus eye Hartline, Wagner and Ratliff (1956) reported that to a first approximation the decrease in the frequency of discharge from an ommatidium caused by a constant inhibitory input is independent of its level of excitation, that is, the value of the inhibitory coefficient is approximately constant irrespective of the excitatory level, e, of the ommatidium.*

The evidence for this is shown in figure 24a. The decrease in the frequency of discharge is plotted on the ordinate as a function of the intensity of the light exciting the ommatidium on the abscissa, for five different intensities of inhibiting illumination - represented by the family of straight lines. Notice that at the higher inhibitory intensities (LOG $I_{INHIB.}$ = 0.0 and -0.5) the decrease in frequency becomes somewhat greater as the exciting intensity (LOG $I_{EXCIT.}$) is increased, whereas the reverse effect occurs at the lower inhibitory intensities (-1.0, -1.5, and -2.0). If the excitatory level of an ommatidium has no effect on its sensitivity to incoming inhibition, then the lines should be

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* The parameter e which appears in equations (1) and (2) is referred at various times as the "uninhibited frequency", "frequency of control", and "level of excitation". Which term is used depends on the context of the statement.

horizontal - that is, have slopes equal to zero. The deviations of the lines from the horizontal are slight and in fact Hartline, Wagner, and Ratliff considered them to be negligible compared to the effects produced by changes in the inhibiting illumination. They concluded therefore that to a first approximation an ommatidium's response to a constant inhibitory stimulus is independent of its level of excitation. This is equivalent to saying that the inhibitory coefficient is independent of the level of excitation of that ommatidium.

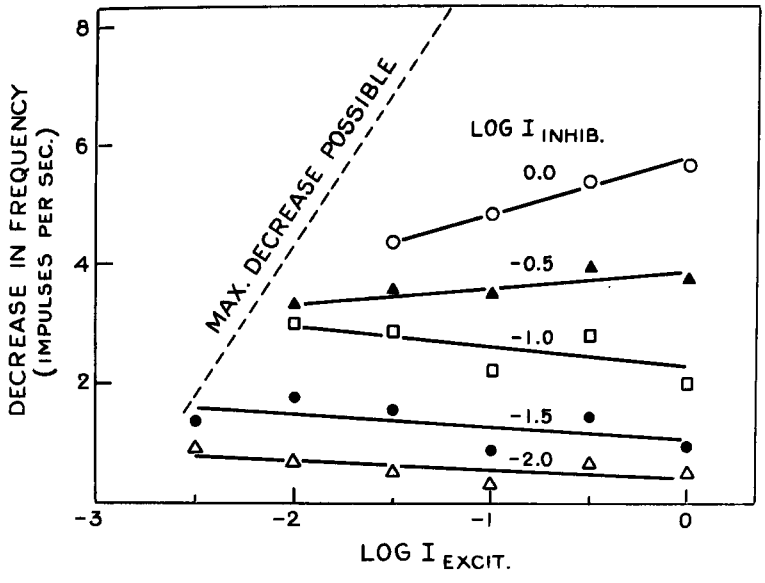
This experiment was repeated by Lange (1965) using antidromic inhibition. Inhibition exerted antidromically destroys the mutuality of the inhibitory interaction and thereby permits a precise control of the incoming inhibition on a particular ommatidium - a control that Hartline, Wagner, and Ratliff (1956) did not have. The results obtained by Lange are shown in figure 24b. The plot is analagous to the previous figure (24a) except that the excitatory and inhibitory inputs are measured in frequency units rather than optical density units. There are definite similarities between these two figures. In both cases the slopes of the lines increase with increasing inhibitory input. The effect, however, is more accentuated in the latter experiment using antidromic inhibition. The positive slopes indicate that the sensitivity of an ommatidium to incoming inhibition is increasing with its level of excitation, and consequently the value of the inhibitory coefficient is also increasing.

To include this effect in the formal description of the inhibitory system, Lange modified the set of simultaneous equations (2) as follows:

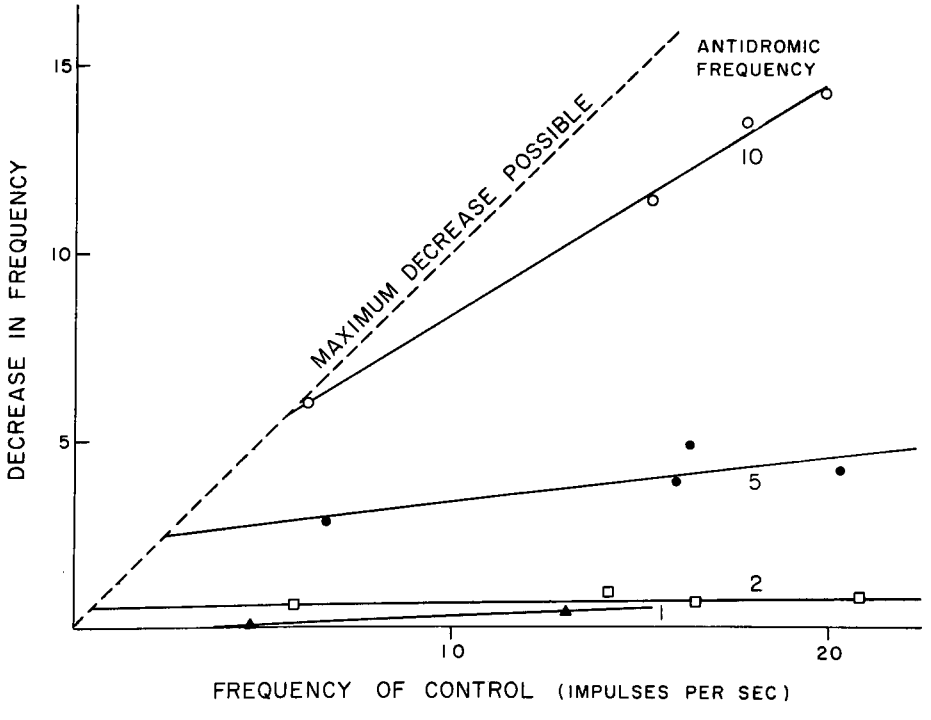
$$r_p = e_p - (1+ae_p) \sum_{\substack{j=1 \\ j\neq p}}^{n} K_{pj}(r_j - r^o_{pj}) \qquad (3)$$

This set of equations differs from (2) by the factor $(1+ae_p)$ which, in effect, "corrects" the value of $\sum K_{pj}$ for each e_p - the level of excitation on the p^{th} ommatidium. From the antidromic experiments

Figure 24. The dependence of inhibition on the excitatory level of an ommatidium. Figure A gives the amount of inhibition (decrease in frequency of discharge) on the ordinate as a function of the intensity of light exciting the ommatidium on the abscissa, for five different intensities of inhibiting illumination. (Logarithmic light intensity units were used to measure both the inhibitory and excitatory inputs.) Data points indicated by the same symbols were all measured with a constant inhibitory light intensity designated by the numbers above each heavy line. Lines with zero slopes indicate that the inhibition is independent of the level of excitation. Figure A from Hartline, Wagner, and Ratliff (1956). Figure B illustrates a similar experiment using antidromic inhibition. The plot in B differs from that in A only by the units of the excitatory and inhibitory inputs: frequency instead of light intensity. Figure B from Lange (1965).


DECREASE IN FREQUENCY
(IMPULSES PER SEC.)
8
6
4
2
-3
-2
-1
0
LOG I EXCIT.
MAX. DECREASE POSSIBLE
LOG I INHIB.
0.0
-0.5
-1.0
-1.5
-2.0

A


DECREASE IN FREQUENCY
15
10
5
10
20
FREQUENCY OF CONTROL (IMPULSES PER SEC)
MAXIMUM DECREASE POSSIBLE
ANTIDROMIC
FREQUENCY
10
5
2
1

B

Lange calculated the value of a to be about 0.3. He noted that the corrections implicit in the data of Hartline, Wagner, and Ratliff (1956) lead to an a of 0.12.

The incorporation of the correction factor, $(1+ae_p)$, into the classical equations (2) does not appear to affect their behavior. At a constant level of excitation the set of equations (3) reduces to (2). Also for any particular level of inhibition, the decrease in frequency, $e_p - r_p$, becomes a linear function of the excitation level with a slope depending on the level of the inhibitory input. Therefore the equations (3) are nonlinear if either the pattern of excitation or the level of inhibition is not specified. The nonlinearity is introduced by the dependence of the inhibitory coefficient upon the level of excitation.

A similar nonlinearity was hypothesized for the locust visual system by Thorson (1965) to help explain optomotor responses in this insect. In a theoretical analysis of inhibition in the mammalian cochlea Furman and Frishkoff (1964) used a similar set of equations to describe backward-shunting inhibition.

The modification of the classical equations by Lange permits a more precise description of the inhibitory system in the Limulus eye. It should be emphasized however that this modification is based on the augmented inhibitory effect that was observed upon increasing the level of excitation from 5 to 20 impulses/sec. Several experiments were carried out for this thesis that extended the range of excitation far beyond 20 impulses/sec. The results of these experiments show that the inhibitory effects which were steadily increasing for $e < 20$ impulses/sec began to diminish for $e > 30$ impulses/sec. One of these experiments will now be described.

The experiment - similar to those in figure 24 - was carried out with e increasing from 2 to 60 impulses/sec for four different levels of inhibition. The large variation in the excitation levels was obtained by using the fiber optics illumination system described in

Chapter II. The experiment employed a fiber optic bundle to illuminate a cluster of four ommatidia as a source of inhibition and a single optical fiber to isolate optically a nearby ommatidium which was used to monitor the inhibition exerted by the cluster. The response of the single ommatidium was recorded together with the response of one of the four ommatidia in the cluster with the assumption that each ommatidium in the cluster responds alike (see Chapter III). The results of the experiment are shown in figure 25. Notice that the inhibitory effects in the region from e = 0 to e = 20 are nearly identical to those in figure 24. Beyond that region, however, the effects increase to a maximum and then decline at the highest excitatory levels. The maximum effects occur between 25 and 30 impulses/sec. Clearly, the inhibitory effect on an ommatidium is not a simple function of the excitatory level of the ommatidium.

To obtain a quantitative relationship between the strength of inhibition - that is the inhibitory coefficient - and the level of excitation, an experiment was performed in which the inhibitory input was held constant while e was varied at random from 5 to 45 impulses/sec. The data from this experiment were used to calculate an inhibitory coefficient for each value of e. The results are shown in figure 26. At the lower values of e (5 to 25) the coefficient increases linearly with increasing e, which agrees with Lange's result. At the higher values of e the coefficient decreases at approximately the same rate. The results from a number of experiments in addition to the one in figure 26 indicate that an ommatidium is inhibited most effectively when its firing rate is about 26 impulses/sec: the effectiveness diminishes for rates higher or lower than this value. This effect can be incorporated into the classical set of equations (2) by a slight modification of Lange's correction factor:

$$r_p = e_p - (1+a|e_p-26|) \sum_{\substack{j=1 \\ j\neq p}}^{n} K_{pj}(r_j - r^o_{pj}) \qquad (4)$$

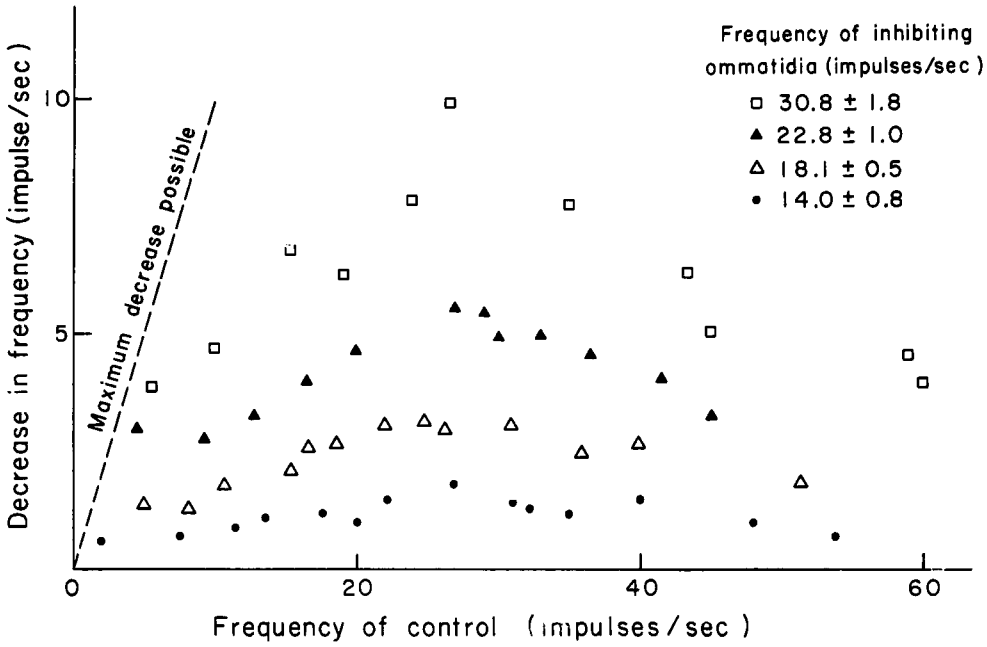


Figure 25. The dependence of inhibition on the level of excitation. This experiment is similar to those in figure 24 with the exception that excitatory input on the ommatidium under observation covers a much wider range; 2 to 60 impulses/sec as compared with 1 to 16 impulses/sec for figure 24a and 5 to 20 impulses/sec for figure 24b. The excitatory and inhibitory light intensities were provided by a fiber optics system. The amount of inhibition is measured on the ordinate and level of excitation on the abscissa. The table gives the response levels on one of a cluster of 4 ommatidia that was used as a source of inhibition.

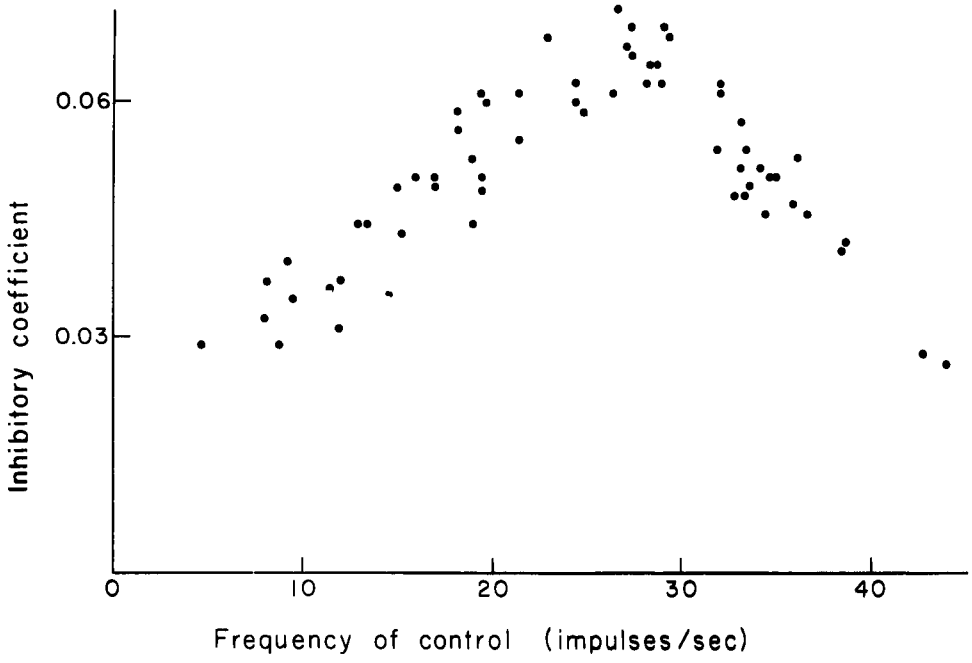


Figure 26. The dependence of the inhibitory coefficient on the level of excitation. The experiment arrangement is the same as that used in figure 25. However, in this experiment the inhibitory input from the cluster of ommatidia was held constant while the level of excitation of the ommatidium under observation was varied at random from 5 to 45 impulses/sec. The data was used to calculate an inhibitory coefficient for each level of excitation (or frequency of control). Each calculation is represented by a point on the graph.

where the correction a now multiplies the absolute value of the difference between the level of excitation e_p and the point of maximum inhibitory sensitivity. From the results in figure 26 the value of a is calculated to be about -0.03 if e is expressed in frequency units. The correction factor is valid for excitatory levels from 5 to 45 impulses/sec.

The degree to which one ommatidium inhibits another depends not only on the distance between them (Chapter III) but also on the activity of both. The excitatory level of an ommatidium effectively determines its sensitivity to incoming inhibition. For the antagonistic influences to interact with one another in this way they must converge to a "summing" point within the ommatidium. From what is known about the electrical properties of the receptor (Purple, 1964) it is very likely that this "summing" point lies within the eccentric cell. Purple (1964) has, in fact, studied in great detail the integration of the excitatory and inhibitory influences in the eccentric cell. He constructed two simple electrical equivalent circuit models of the eccentric cell. It is interesting to note that the general features of the family of curves in figure 25 are predicted by the particular model which considers excitatory and inhibitory influences acting at the same point within the cell. Based on Purple's calculations the increase in the inhibitory effect with increasing excitation and then the eventual diminution of the effect may be interpreted as the result of two competing mechanisms which control the magnitude of the inhibitory current at the summing point: (1) the potential driving force due to the difference between the membrane and inhibitory potentials and (2) the shunting of the inhibitory current through other parts of the cell due to the general decrease in membrane resistance upon excitation (for a more detailed discussion see Purple, 1964).

Whatever the cellular mechanisms may be it is clear that the integration of the excitatory and inhibitory influences produce significant nonlinear effects. It is important in certain experiments

on the Limulus eye to be constantly aware of these effects. For example, in the mapping experiments (Chapter III) the level of excitation of each ommatidium in the "mapping field" must be kept the same. If not, the variation of the inhibitory coefficient will depend on two parameters: the position in the field and the excitatory level. As mentioned in Chapter II the excitatory level of each mapped ommatidium was adjusted near to the point of maximum sensitivity: 26 impulses/sec.

The dependence of the inhibitory coefficient, K, upon the level of excitation, e, may have some important effects on the response of the eye to various patterns of illumination, both stationary and moving. For example, the diminution of K for low values of e (see figure 26) appears to increase the functional range of an ommatidium by decreasing its sensitivity to incoming inhibition when its firing rate is very low. In other words, an ommatidium can respond at a finite rate under inhibitory influences that would have decreased its firing rate to zero if K did not diminish at low e.

This effect was investigated theoretically by applying the correction factor in equation (4) to the computer model of the inhibitory system (the model was developed by Quarles and is described in Chapter IV). The response of the model was computed for several step-patterns of excitation in which the height of the step, Δe, was held constant at 10 impulses/sec while the levels of excitation on either side of the step were varied. The computed response patterns show two striking effects: (1) the difference between the steady response levels on either side of the step decreases as the excitatory levels decrease with $\Delta e = 10$ impulses/sec and (2) the resulting Mach bands show asymmetries that depend on the excitatory levels.

More precisely, in (1) the difference in the steady response levels - far from the step - is 8.5 impulses/sec for a step in excitation from $e = 26$ to $e = 36$ impulses/sec, whereas the difference

is 4.3 impulses/sec for a step from e = 16 to e = 26 impulses/sec. The difference is less for steps ($\Delta e = 10$) with lower excitatory levels. Apparently, at low levels of incident illumination the visual system neglects the absolute light levels for the purpose of preserving information on the more significant features of the visual image: contours and borders. It is well-known that these features are enhanced in the neural response patterns by the appearance of Mach bands, however, it is obvious that no Mach bands would appear if the responses were zero. It seems therefore that the ability of an ommatidium to vary its sensitivity to inhibition according to its excitatory level allows the visual system to transmit meaningful information to the central nervous system over wide ranges of incident light intensity.

The other effect (2) is concerned with the size and shape of the Mach bands appearing in the computed response patterns. It was found that a step-pattern of excitation which is symmetric about 26 impulses/sec - for example a step from e = 21 to e = 31 impulses/sec - produced Mach bands that are symmetric or more precisely that are inverted images of one another. An equivalent step in excitation ($\Delta e = 10$ impulses/sec) above or below 26 impulses/sec will produce asymmetries in the computed Mach bands, that is the amplitudes of the Mach bands (see Chapter IV) appearing on either side of the step are not equal. This is understandable because the K's are equal for e's that are symmetric about 26 impulses/sec, whereas this is not the case for e's that are distributed unequally about the point corresponding to the maximum value of K.

It should be apparent from the foregoing discussion that the dependence of K upon e can modify substantially the response of the eye to simple stationary patterns of illumination. In addition, there is good reason to believe that the responses to moving patterns will be similarly affected; however, there is as yet no experimental or theoretical evidence on this point and any discussion would be purely speculative.

Presumably, for various adaptive reasons, the property of lateral inhibition "tunes" the eye to particular characteristics in the visual field. The main features of this "tuning" or selective property are adequately described by the classical Hartline-Ratliff equation (2), however, the more subtle features described in this appendix require a somewhat modified version (4). It is entirely possible that, in the light of future experiments, the latter system of equations will itself have to be modified to account for new "subtleties".

APPENDIX II

THE PHYSIOLOGICAL RANGE OF THE OMMATIDIA IN THE *LIMULUS* EYE

Direct sunlight is 10^{14} times as intense as the light coming from the faintest star. Both are visible to the human eye. This extremely large range in sensitivity is primarily the result of a dual receptor system: the cones which operate at high light intensities, and the rods which function at low light intensities. The *Limulus* lateral eye, however, contains only one type of receptor - the ommatidium - and consequently the range in sensitivity of this eye is somewhat restricted compared to the human. The evidence discussed below indicates that light which is more than 10^{10} times as intense as the threshold illumination of an ommatidium is probably of little "use" to the organism. Evidence for the faintest light intensity will be discussed first.

For ommatidia in the *Limulus* eye the criterion for "seeing" is the discharge of optic nerve impulses in response to a brief flash. Invoking this criterion, Hartline, Milne, and Wagman (1947) determined that the probability of "seeing" at threshold follows the Poisson distribution. Their analysis was very similar to the classical threshold experiments on the human eye by Hecht, Schlaer and Pirenne (1942). It should be noted that under the conditions of these experiments the probability of seeing at threshold is not an expression of the variability of the receptor mechanism but rather of the variability of the number of quanta in a series of short flashes. By matching the experimental frequency-of-"seeing" curves to the Poisson probability distributions Hartline, Milne, and Wagman found that in the most sensitive units at least two excitatory events - perhaps caused by the absorption of two quanta - were required in an ommatidium to initiate one or more impulses in an optic nerve fiber. The results of the experiments by Hecht, Schlaer, and Pirenne indicate that at least five quanta must be absorbed at the human

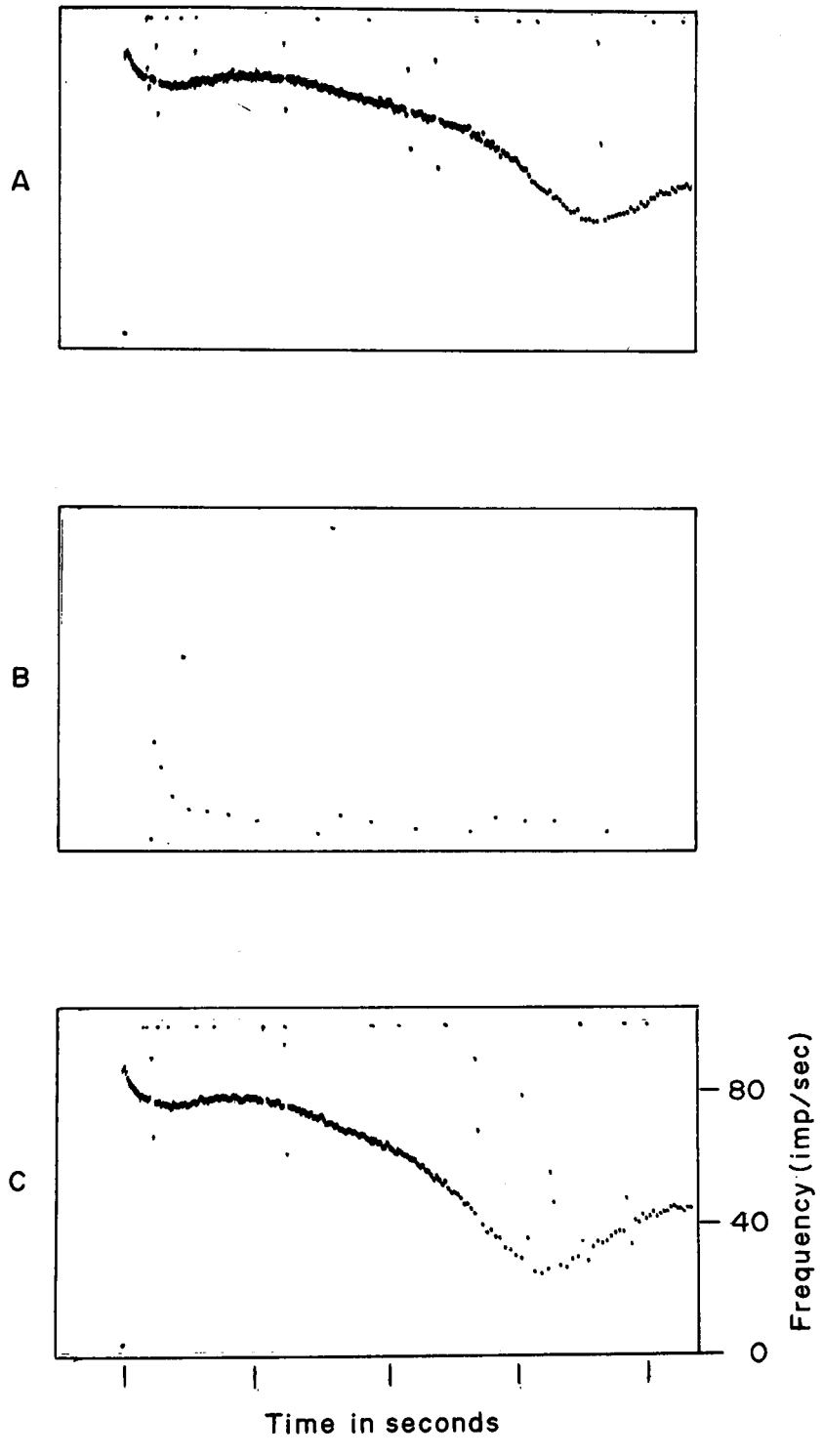
retina to produce a visual impression. In either case the amount of light absorbed by the photoreceptors in a near-threshold flash is quite small - so small that only a few quanta are involved.

Due to the reflections and scatter in the dioptric apparatus of the eye, the number of quanta incident on the cornea in a near-threshold flash is much greater than the number incident on the receptor layer. To initiate one or more optic nerve impulses from the most sensitive dark-adapted ommatidium approximately 10^4 quanta/flash are required at the cornea (Hartline, personal communication). This value will be taken as a rough estimate of the maximum sensitivity of the receptor unit; that is, one or two quanta absorbed for every 10^4 quanta incident on the ommatidial facet. Consider now the evidence for the upper limit of the maximum "useful" light intensity.

As it was pointed out in Chapter II, fairly intense light from a single optical fiber can illuminate the facet of a single ommatidium with a minimum amount of scatter to its neighbors. With the standard illumination system described in Chapter II, the maximum intensity of visible light transmitted by the single fiber was 2.4×10^{14} quanta/sec. It is often found that incident light of this intensity can temporarily "knock-out" the function of an ommatidium, indicating that intensities equal to or greater than this value are beyond the physiological range of the receptor.

This effect is demonstrated by the series of records in figure 27. For this experiment an ommatidium was optically isolated with a single optical fiber using the techniques outlined in Chapter II. The optic nerve fibers from this ommatidium and one of its nearest neighbors were placed on recording electrodes. The response of the neighbor was used to monitor the quality of optical isolation of the other ommatidium. The eye was completely dark-adapted (40 minutes) and then the maximum intensity light was turned on for 6 seconds. The first 4.5 seconds of the response is shown in record A which plots the "instantaneous" frequency of the combined discharge of the two

Figure 27. The effect of intense light on the responsiveness of an ommatidium. Each of the three records is an "instantaneous" frequency plot of the discharge of impulses from two optic nerve fibers recorded simultaneously. The points correspond to the reciprocal of the interspike intervals and are plotted in frequency units on the ordinate as a function of time on the abscissa. The two nerve fibers represent neighboring ommatidia on the eye. One of the ommatidia was "optically isolated" with a single optical fiber (see Chapter II). Record A gives the first 4.5 seconds of the response of the dark-adapted ommatidia to 6 seconds of the maximum light intensity (2.4×10^{14} quanta/sec) transmitted by the optical fiber. The predominant response (continuous curve) is that of the optically isolated ommatidium. The points lying on either side of the continuous curve represent the few impulses fired by the neighboring ommatidium in response to scattered light. The same stimulus was repeated 30 seconds later. The first 4.5 seconds of the response is shown in Record B - notice that only the neighbor responded. Five minutes later the light was turned on again for 6 seconds with the same maximum intensity. Record C gives the response to this last stimulus.


A
B
C
80
40
0
Frequency (imp/sec)
Time in seconds

nerve fibers on the ordinate as a function of time on the abscissa. Each point in the record corresponds to the reciprocal of the interspike interval. The principal response pattern - continuous curve - was elicited by the optically isolated ommatidium. The points that do not lie on this curve represent the impulses fired by the neighbor in response to scattered light. However, the scattered light was small; in record A the optically isolated ommatidium fired approximately 300 impulses while its neighbor only fired 16 impulses.

Thirty seconds after record A the light was turned on again for 6 seconds with the same maximum intensity. The first 4.5 seconds of the response is shown in record B. The 16 points in record B correspond to the impulses fired by the neighbor. The optically isolated ommatidium did not fire an impulse over the entire period of illumination - its function was apparently "knocked-out" by the intense illumination that was used to obtain record A. More precisely, the receptor was light-adapted to the extent that it would not respond to the same intense illumination 30 seconds later. The fact that the neighbor fired the same number of impulses in records A and B indicates that the stimulus was the same in each case. The normal light sensitivity of the ommatidium returns after a short period in darkness as demonstrated by record C which was taken 5 minutes after B.

A dark-adapted ommatidium therefore responds vigorously to intense illumination, which if it is high enough renders the ommatidium unresponsive to subsequent stimulation; the normal response returning after a short period of time in the dark. For this reason it is suggested that light of this intensity (10^{14} quanta/sec) or greater maybe of little "use" to the organism. To obtain the effect illustrated in figure 27 the total amount of light incident on the facet of the ommatidium was 10^{15} quanta. A rough calculation of the number of these quanta absorbed by the ommatidium is made by multiplying this value with the calibration factor determined by Hartline: 10^{15} incident quanta x 1 absorbed quanta/10^{4} incident quanta $\simeq 10^{11}$ absorbed quanta. The absorption by the photosensitive structure of a few quanta to roughly 10^{11} quanta gives an estimate of the functional or physiological range of the receptors in the Limulus eye.

APPENDIX III

THE PROJECTION OF THE OPTIC NERVE ON THE RETINAL MOSAIC

In dissecting the optic nerve in Limulus, it is common to find that the nerve trunk divides naturally into a half-dozen or so subunits which are more difficult to split. It has been observed by F. Dodge and B. Knight (personal communication) that the nerve fibers contained within these subunits project to ommatidia that are associated in horizontal strips on the eye, that is, strips that are parallel to the antero-posterior axis of the animal.

Several experiments were performed to investigate this property in greater detail. For these experiments a short length of the optic nerve (1 cm.) immediately behind the eye was separated into its natural subunits. The subunits were placed one-by-one on a recording electrode and the eye was searched with a single optical fiber (described in Chapter II). With this method it was possible to determine the approximate location on the eye of the ommatidia which when illuminated discharged impulses in the nerve fibers of each subunit.

The results from one of these experiments are illustrated in figure 28. In this particular experiment the nerve trunk divided "naturally" into seven subunits. The numbered areas on the eye represent the projection of each subunit. The cross-hatched areas correspond to the regions in which no activity was found in any of the subunits. (It is possible that the nerve fibers, emanating from the ommatidia in these regions, were damaged during the dissection.) Notice that all of the subunits project to areas that are more or less elongated in the antero-posterior direction. Subunit number 5 stretches across the entire eye; whereas, subunits 3 and 4 appear to overlap one another. It should be emphasized that the precise configuration of these projections varies from eye to eye. For example, the location of two overlapping areas (3 and 4) in the dorsal part of

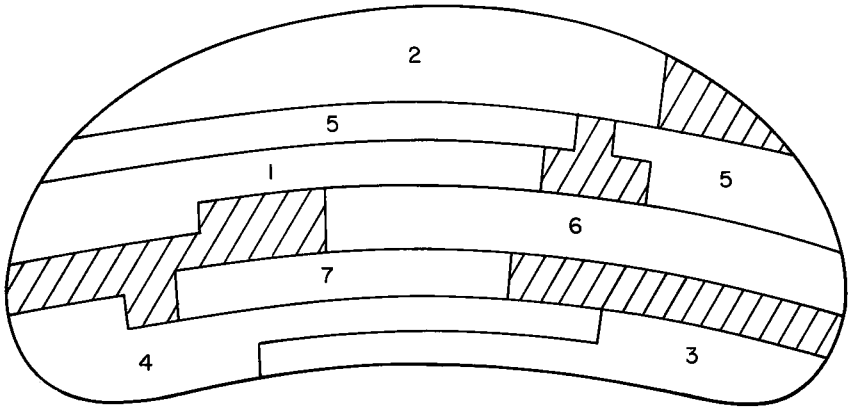


Figure 28. The projection of "natural" subunits of the optic nerve on the retinal mosaic. The heavy line represents the perimeter of the eye with the dorsal direction down and the anterior direction to the left. Each numbered area corresponds to the general location on the eye of the ommatidia whose nerve fibers were contained within seven natural subunits of the optic nerve. The cross-hatching corresponds to areas from which no response to illumination was recorded in any one of the seven subunits. The numbered areas were mapped out by placing each subunit one by one on a recording electrode and searching the eye with a fiber optic light source.

the eye was not found in the other experiments.

In several experiments the subunits themselves were divided into smaller components. The subunits, however, do not appear to divide "naturally" as does the main nerve trunk. Using the same methods described above, the projections of nerve fibers in the smaller components were determined. Since a relatively small number of fibers are contained within each component it is possible to map the projection of the component to individual ommatidia instead of to a general area on the eye as the previous experiment.

The results from one experiment are shown in figure 29. Each circle represents an ommatidium and the ones containing numbers represent the ommatidia whose responses were detected in each of the 6 smaller components. The random distribution of ommatidia whose responses were not recorded (indicated by the open circles) suggests that their nerve fibers were damaged during the dissection of the subunits. Notice that the ommatidia corresponding to the smaller components are associated more or less in horizontal strips. This is certainly true for components 1, 3, 4, and 5; whereas, component 2 contains too few active fibers to tell, and component 6 contains too many.

It is evident from these results that the optic nerve fibers within the main nerve trunk divide naturally into six or seven subunits and the fibers within these subunits project to ommatidia located in horizontal strips on the retinal mosaic. In histological studies of the cross-section of the optic nerve trunk Nunnemacher (personal communication) found that the many fibers within the trunk are grouped into small bundles and that each bundle is separated from the others by a sheath of Schwann or satellite cells. In addition, the small bundles appear to be divided into a half-dozen or so large groups. Perhaps these large groups correspond to the natural subunits that are observed upon dissection of the nerve trunk.

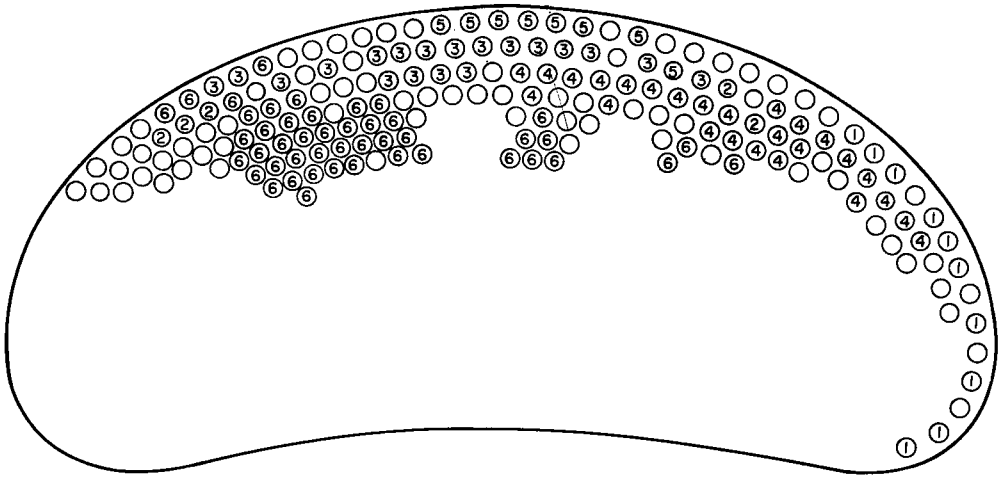


Figure 29. The projection on the eye of smaller components of a natural subunit of the optic nerve. The outline of the eye is illustrated with the dorsal direction down and the anterior direction to the left. The circles represent the approximate location on the eye of some of the ommatidial facets. The numbered circles correspond to ommatidia whose nerve fibers were contained within six small components of a subunit of the optic nerve. Each number corresponds to one of the components. The projection on the eye of a particular component was determined by exploring the corneal facets one by one with a fiber optic light source while at the same time recording the impulses discharged by the nerve fibers within the component. Illuminating the facets represented by the open circles produced no discharge of impulses in any one of the six components. Different eyes were used for the experiments illustrated in figures 28 and 29.

Near the central ganglion the optic nerve appears to divide into several bundles which fan out and enter the optic lobe of the ganglion at various points. Some very preliminary observations by R. Shapely (personal communication) suggest that the nerve fibers within these bundles also project to horizontal strips on the eye. From this observation it would appear that the integrity of the bundles, emerging from the eye, is maintained down the entire length of the optic nerve to the central ganglion. If this is true, then the optic nerve may project horizontal "slices" of the retinal image to particular locations in the ganglion.

This peripheral property of the Limulus visual system becomes all the more interesting when one considers the elliptical configuration of the ommatidial inhibitory field (see Chapter III). Recall that the major axis of the asymmetric field lies in the horizontal direction; thus, the bulk of the inhibitory influences from any given ommatidium is exerted in this direction. The striped projection of the optic nerve on the eye corresponds to the long axis of the inhibitory field, making it possible for the subunits of the optic nerve to send information to common or nearby points in the central ganglion from ommatidia that inhibit one another over considerable distances.

Add these properties to those discussed in Chapters I and III of this thesis and it becomes apparent that the so-called "simple" visual system of Limulus is in fact a highly sophisticated one that undoubtedly has evolved to select from the environment certain information which is essential to the livelihood of the organism. Hopefully, in the future when something is known about the important visual cues in the animal's natural habitat and about the elementary visual data processing in the central ganglion, it may be possible to determine the functions of the various properties in what is most likely an elegant system for selective pattern recognition.

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