CHEMICAL MODIFICATIONS TO TRANSDUCTION
IN AN INSECT EYE

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DECLARATION

The reprint that is bound into chapter five describes work performed in collaboration with Jo Howard. The electron micrographs in chapter one were prepared by Ms Jadwiga Duniec. Apart from these inclusions, I declare the rest of the work described in this thesis to have been my own.

Richard Payne

Material in the following chapters has been published:

Chapter two

Chapter three

Chapter four

Chapter five

A manuscript based on the work in chapter 3 has been submitted to the Journal of Physiology (Lond.)
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Almost all of the members of the Department were sooner or later roped into reading the seemingly endless drafts of my papers and of this thesis. I thank this multitude for their patience and criticism.

Mike Savage, Roger Welsh, Kerrie Ruth and Kevin Downing administered technical support and light relief. David Sandilands ran the central computer. Bruce Ham bred the animals.

Tess Falconer and Pam Coote typed the first drafts of most of the chapters of this manuscript.
ABSTRACT

This thesis examines several aspects of the electrophysiology, pharmacology and kinetics of transduction by photoreceptors in a superfused retina of a locust compound eye.

Using intracellular theta-glass electrodes and a current clamp device, the basic electrophysiology of the photoreceptors is examined in chapter 1. Light depolarizes the photoreceptors' plasma membrane through the action of an increased membrane permeability, primarily to sodium ions. The rectification properties of the membrane and a prolonged light-induced increase in the potassium conductance of the membrane antagonize the effect of the light-activated sodium conductance. The response of the photoreceptor to steady illumination is accompanied by voltage noise that appears to be caused by fluctuations in the light-activated sodium conductance.

Chapters 2, 3 and 4 demonstrate that several chemical agents are, like light, able to cause a noisy depolarization of the photoreceptor. Treatment with sodium azide (1 mM) or sodium fluoride (10 mM), followed by a series of light flashes results in a maintained, noisy depolarization of the photoreceptor. This depolarization appears to develop from afterpotentials that follow the response to each light flash. Fluoride, unlike azide, acts irreversibly.

Neutral anaesthetics such as chloral hydrate are also able to initiate a noisy depolarization of the photoreceptor. The action of chloral hydrate is very rapid and requires no stimulation by flashes of light. Depolarizations of up to 15 mV can be produced within seconds of the application of chloral hydrate to the retinal surface.
The voltage noise that accompanies the depolarization produced by these chemicals mimics the voltage noise that can be recorded during steady illumination of the photoreceptor by strong light. The properties of the chemically-induced voltage noise are consistent with the proposal that it arises from the spontaneous activity of an intermediate at a late stage in phototransduction, occurring after the stage of biochemical amplification.

In chapter 5, the average response of the photoreceptor to a brief, weak flash of light (the 'impulse response') is investigated. The impulse-response of locust photoreceptors cannot be fitted with models of phototransduction that involve cascades of first order chemical processes. Too many stages are required in the cascades and the time-constants for each stage would have too great a variability from cell to cell. A simple alternative model of visual transduction is proposed which involves a threshold response of ionic channels to a chemical transmitter whose concentration rises inside the receptors in the physical locality of a bleached rhodopsin molecule.

Changes in the temperature of the photoreceptors and changes in their state of light-adaptation produce simple changes in the time-scale of the impulse-response.
<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GENERAL INTRODUCTION</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
</tbody>
</table>

**CHAPTER 1 -- The electrophysiology and anatomy of perfused locust retinula cells**

- **Summary** 13
- **Introduction** 15
- **Methods** 17
- **The electrophysiology** 21
- **The anatomy** 36

**CHAPTER 2 -- Suppression of noise in a photoreceptor by oxidative metabolism**

- **Summary** 42
- **Introduction** 42
- **Methods** 42
- **Results** 43
- **Discussion** 47

**CHAPTER 3 -- Fluoride blocks an inactivation step of transduction in a locust photoreceptor**

- **Summary** 50
- **Methods** 52
- **Introduction** 53
- **Results** 56
- **Discussion** 69

**CHAPTER 4 -- Neutral anaesthetics induce a rapid, noisy depolarization of locust photoreceptors**

- **Summary** 75
- **Introduction** 76
- **Results** 78
- **Discussion** 85
CHAPTER 5 -- Modelling of the impulse-response of locust photoreceptors

Summary 88
Introduction 89
Results 94
Discussion 99

GENERAL DISCUSSION 103

REFERENCES 112
GENERAL INTRODUCTION TO THE THESIS
GENERAL INTRODUCTION

'Transducer noise' and ionic channels

To obtain reliable visual information about a scene, the nervous system requires a photo-transducer that converts light quanta into an electrical signal with high fidelity. In order to convey the maximum amount of information, the ideal transducer should be noise-free, with as flat a frequency response as possible and should respond with no latency. Ideal transduction of this kind is, inevitably, unattainable by biological transducers.

Locust photoreceptors, therefore, cannot be expected to meet the exacting requirements set for an ideal transducer. The photoreceptor is capable of amplifying the energy of a photon up to one million times so as to produce a detectable electrical event. The high amplification performed by the photoreceptor results in a response to each photon of light that is 20 times higher than background noise (Lillywhite, 1977). However, significant noise is injected into the response of the cell by the transduction apparatus and has been termed 'transducer noise' (Lillywhite and Laughlin, 1979). The photoreceptor also has a poor high-frequency response, resulting in 'motion blur' which limits the eye's ability to resolve visual scenes that move across the eye at high speeds [greater than 200 cycles per degree (Srinivasan and Bernard, 1975; Howard, 1981)]. The latency of the photoreceptor's response (10 ms in the light-adapted state and 30ms when dark-adapted) means that the neural image of scenes moving at such speeds will lag behind its true position in space by as much as 12 degrees, resulting in further distortion of the visual scene (Howard, 1981).
Very little is known about the mechanisms that limit the response of the photoreceptor to high frequencies of stimulation and produce the latency associated with phototransduction, or about those that generate the transducer noise. A full understanding of the mechanism of phototransduction will lead to an insight into the compromises that have been made in the design of biological transducers with such high amplifications as those possessed by the photoreceptors of several invertebrate species.

Detailed knowledge of transduction in invertebrate photoreceptors has been confined, largely, to the first and last steps - the photo-isomerization of rhodopsin and the changes in the electrical conductance of the receptor's membrane that are directly responsible for the changes in electrical potential that constitute the signal of the cell.

The primary event in phototransduction is the photo-isomerization of a molecule of the visual pigment, rhodopsin. In invertebrate photoreceptors, the rhodopsin is converted to another pigment, metarhodopsin, which may be photo-isomerized back into rhodopsin, usually by light of a different wavelength from that which excites the forward reaction (review; Hamdorf, 1979). The recent work of Hamdorf and Kirschfeld (1980) suggests that, in fly photoreceptors, rhodopsin is first rapidly photo-isomerized to a short-lived, intermediate form of metarhodopsin which is stable for 3-5ms at 10°C and which irreversibly initiates further transduction processes as it decays into stable metarhodopsin. At 10°C, the electrical response of the cell to a flash of light lasts for over 100ms, the latency of the response to a very bright flash is approximately 9ms, and the response to a dim flash has a latency of 70ms. Thus while the time taken for conformational changes in the visual pigment may account for some of
the response latency, its contribution to the latency of response to a
dim flash, and to the duration of the response to a flash of any
intensity, is small.

Processes within the isomerized pigment also cannot account for
the spatial extent of activation by light. Brown and Coles (1979)
estimate that a single photon is able to activate an area of cell
membrane several microns in diameter within 100 ms of the delivery of
a flash. The current upper estimates for the lateral diffusion of
rhodopsin molecules in invertebrate photoreceptive membranes
(Goldsmith and Wehner, 1977; Almagor et al, 1979) appear to rule out
the possibility that rhodopsin could diffuse over this distance in the
required time. It therefore appears necessary that there be some
'internal transmitter' (Cone, 1973) which is released by activated
pigment and which excites a patch of membrane in the locality where
the photo-isomerization occurred. It is to the kinetics of the release
of this transmitter, and to the kinetics of its interaction with the
receptors in the membrane that control the membrane's conductance,
that we should look for an explanation for the latency and noise
inherent in phototransduction.

The final events in transduction, the changes in the electrical
conductance of the cell membrane that are directly responsible for the
changes in electrical potential, are well understood in some
preparations. Both barnacle photoreceptors and those on the ventral
nerve of the horseshoe crab, Limulus, have been analysed using the
voltage clamp technique (Millecchia and Mauro, 1968; Brown et al,
1970; reviews of barnacle data in Laughlin, 1980 and of Limulus data
Most invertebrate photoreceptors depolarize in response to light, but most do not produce action potentials (review; Shaw, 1978). In Limulus photoreceptors, the depolarization that is activated by light is caused by an increased permeability to sodium ions, which constitutes the 'light-activated' conductance. The depolarization caused by the inward sodium current is modified by the action of outward potassium currents produced by conductances that are under the control of both the membrane potential and light.

Chapter one of this thesis presents experiments that show that the light—induced depolarization of locust photoreceptors may, similarly, be due to a primary light-activated sodium conductance, coupled with voltage- and light- dependent potassium conductances.

Recently, many authors have begun to apply the concepts of ionic channels to the light-activated conductance. Channels are gated pores that are thought to mediate ionic permeabilities across other excitable membranes (review; Neher and Stevens, 1977). The proposed internal transmitter may be regarded as a messenger that opens sodium channels in a similar manner to synaptic transmitters (review: Steinbach and Stevens, 1976). The kinetics of phototransduction have been interpreted in terms of the kinetics of the release of the internal transmitter and of its interaction with membrane receptors, so as to open ionic channels (e.g.Cone, 1973; Wong, 1980; Fain and Lisman, 1981). The present thesis will also, on a number of occasions, interpret experimental findings in terms of the 'channel hypothesis'.

An understanding of the kinetics of phototransduction is, perhaps, best approached through a study of the kinetics of the quantal event, the result of the absorption of a single photon.
At very low light intensities, we can study the quantal events directly, provided they are of a large enough amplitude. Quantal events ('bumps') recorded from locust photoreceptors arise after a variable latency, between 10 and 100ms, and have amplitudes that might vary from 1 to 7 mV in any one receptor (Lillywhite, 1977). It is the variation in bump amplitudes that causes most of the transducer noise described by Lillywhite and Laughlin (1979). Therefore, like the bumps recorded from other invertebrate photoreceptors, locust bumps appear to arise from a random process.

At higher intensities of light, the bumps fuse to form a graded receptor potential (Scholes, 1964). The depolarization induced by steady illumination is accompanied by noise (light-induced noise) that is due to the random occurrence and amplitudes of the bumps that sum to produce the light-induced depolarization. The light-induced noise has two components: 'Photon shot noise', and transducer noise. Photon shot noise arises from random fluctuations in the number of bumps occurring in a period of time, due to the statistics of photon release from sources of light with a constant mean emission. Transducer noise, arises from fluctuations in the size of the bumps.

Noise which arises from the independent summation of 'shot events', can be analysed so as to extract the amplitude and time-scale of the events, as well as their rate of occurrence (e.g. Katz and Miledi, 1972). A knowledge of the power spectrum and variance of the noise is required. This method was first applied to light-induced noise recorded from Limulus lateral eye photoreceptors by Dodge et al (1968) and the analysis has been extended to Drosophila photoreceptors by Wu and Pak (1978) and to Limulus ventral and lateral eyes by Wong and collaborators (Wong, 1978; Wong et al., 1980). A critical review of some of this work may be found in Laughlin (1980) and Fain and
Lisman (1981). As Laughlin points out, a problem with the work, so far, has been the failure to recognize the importance of transducer noise which, in dark adapted locust photoreceptors, contributes as much to the total noise variance as does photon shot noise (Lillywhite and Laughlin, 1979).

Despite their limitations, the results so far obtained from noise analysis are probably qualitatively valid, and some interesting facts have begun to emerge. Both the amplitude and the duration of the bumps appear to decrease at high light intensities. Wong (1978), working on the ventral photoreceptors of Limulus, interprets these changes as a decrease in the number of ionic channels opened by each photon. At low light intensities, when the cell is dark-adapted, each effective photon may open as many as 1000 sodium channels, as was inferred by Cone (1973). This concerted opening of many channels could arise from the release of a 'puff' of transmitter. The mechanism that releases transmitter therefore acts as a biological amplifier. At high light intensities, when the cell is very light-adapted, each photon may open only one sodium channel, or none at all. (Wong 1978).

We can now construct a hypothetical scheme for light-adaptation, using the concepts of ionic channels and of the internal transmitter. As was mentioned above, the light-induced noise contains both transducer noise and photon shot noise. According to the channel hypothesis, the transducer noise arises from the stochastic nature of the interactions between transmitter and channels and also from the kinetics of the release of the transmitter. Wong's hypothesis of light adaptation predicts that, when the cell is dark-adapted, many molecules of transmitter will be released per photon, so as to open many channels. The mechanism that releases transmitter will therefore generate most of the transducer noise, due to variations in the
number of transmitter molecules released per photon. Photon shot noise will also produce large variations in the transmitter concentration in the dark-adapted state, because each photon contributes many molecules to the transmitter pool. The amplitude of light-induced noise in the dark-adapted state will therefore be determined by large contributions from photon shot noise and the noise of the transmitter-release mechanism. Channel noise will not contribute significantly to light-induced noise recorded from a dark-adapted photoreceptor. The time-course of the bump (and hence of the light-induced noise) will be determined by both the transmitter-release mechanism and by the time for which channels remain open.

As the intensity of illumination is increased, and the photoreceptor becomes light-adapted, the relative contribution of photon shot noise and the noise of the transmitter-release mechanism will decline and the noise due to transmitter-channel interactions will become more important. This is because, when the photoreceptor is light-adapted, each photon is presumed to release a much briefer burst of transmitter, containing many fewer transmitter molecules than does the burst produced when the cell is dark-adapted. The duration of the bump is now limited by the time for which an ionic channel remains open (Wong, 1978), so that the time course of the light-induced noise resembles that due to the random opening of single ionic channels. Because of the reduction in the amplification of the mechanism releasing transmitter, there will be less variation in the amount of transmitter released per photon, so that the relative contribution of the release mechanism to transducer noise will decline. Photon shot noise will also become less significant than in the dark-adapted state because less transmitter is released per photon. The amplitude of the light-induced noise at these light intensities will therefore be determined largely by the amplitude of the noise generated by
transmitter-channel interactions, but it may also contain a
collection from photon shot noise. The relative contribution of
photon shot noise will continue to decline as the light intensity is
increased still further until, when the response of the cell is
completely saturated, only channel noise is present. Fain and Lisman
(1981) make this point in their review.

The channel hypothesis, outlined above, is the framework within
which the results of chapters 2, 3 and 4 of this thesis are
interpreted. The basic observation of these chapters is that a variety
of chemical treatments cause a noisy depolarization of the
photoreceptor. As we have seen, the transduction mechanism is known to
generate noise in the light-induced depolarization. Were the
depolarizations induced by the chemicals used in this thesis to be
accompanied by no noise at all, we would have good reason to doubt
that they arose from spontaneous activation of the transduction
mechanism. The observation of the noise provided the stimulus for a
detailed investigation.

**Experimental approaches to transduction**

There are, currently, several approaches to the study of
phototransduction which have been applied in both the vertebrate and
invertebrate fields.

**Kinetic analysis**

Kinetic analysis of the photo-response, in order to model the
elements within the transducer, began as soon as the first reliable
recordings of the response become available. DeVoe (1962, 1963) and
Fuortes and Hodgkin (1964) modelled the photo-response as a linear
cascade of exponential delays. Borsellino and Fuortes (1968) and
Tiedge (1981) have attempted stochastic solutions to the cascade
model, which has been biochemically realised as an enzyme cascade (Borsellino, Fuortes and Smith, 1965). Srebro and Behbahani (1971) and Kramer (1978) have proposed alternatives to the enzyme cascade.

A major problem, for those who model phototransduction, has been the lack of accurate data on the kinetics of the response in the photoreceptors of a variety of species, so that a choice between possible transduction models can be made.

In chapter 5 of this thesis, the average response of a locust photoreceptor, in its linear range, is accurately measured. The effect on the response of changes in the adaptation state of the receptor and in temperature are measured, in an attempt to parallel the work done by Fuortes and his collaborators on Limulus photoreceptors. The deficiencies of the enzyme cascade model in fitting the response of a locust photoreceptor are made clear in this chapter, and a simple alternative model is introduced. The new model is intended as a basis for a quantitative comparative study of the kinetics of phototransduction.

The pharmacological approach

Broadly speaking, this approach involves the introduction into the cell of natural or artificial compounds that modify transduction. Over the past 20 years, research into the nature of ionic channels, and the transmitters that control their opening at synapses, has benefited greatly from the discovery and use of specific neurotoxins (Ritchie, 1979; Steinbach and Stevens, 1976). The discovery that tetrodotoxin specifically blocked the sodium conductance in axons, whereas tetraethylammonium ions specifically blocked the potassium conductance, provided pharmacological evidence that the two conductances were mediated by two separate populations of pores in the
membrane.

Encouraged, perhaps, by these successes, workers in the field of phototransduction have attempted a similar analysis of the pharmacology of the light-activated channels. With the notable exception of the work done on the role of intracellular calcium (review; Stieve, 1981), the results have been disappointing. No pharmacological agent has yet been found that specifically blocks the light-activated conductance (Fain and Lisman, 1981).

Calcium is thought to be the intracellular transmitter that mediates the reduction in the sensitivity of the arthropod photoreceptor caused by light-adaptation. In Limulus photoreceptors, calcium ions are known to be released into the cell's cytoplasm during light-adaptation (Brown and Blinks, 1974), and a raised intracellular calcium concentration is known to reduce the light-activated conductance increase (Brown and Lisman, 1975). Thus calcium may mediate the reduction in the number of channels opened by a photon when the photoreceptor is light-adapted. The use of pharmacological agents, such as the calcium buffer EGTA, have played a major part in these discoveries.

In contrast to the success with the mechanism of adaptation, no agonists of the 'internal transmitter' or blockers of the light-activated sodium channels have, so far, been discovered. Neurotoxic venoms that specifically affect phototransduction are unlikely to exist, blinding being a rather ineffective means of immobilizing prey at close quarters! One is left with a bewildering array of specific and non-specific neurotoxins, with no guarantee that any of them will have an effect. One way of systematically approaching this problem is to first study the effects of inhibitors that modify a broad range of biochemical processes and then to use the results to
make a more informed guess at which specific inhibitors to try.

Chapters 2, 3 and 4 of this thesis examine the effects of two classes of non-specific toxins on the electrophysiology of locust photoreceptors. Chapters 2 and 3 examine the effects of the anionic, metabolic inhibitors, azide and fluoride while chapter 4 examines the effects of neutral anaesthetics. Both classes of toxins produce noisy depolarizations of the cell and, in chapter 3, an attempt is made to confirm the hypothesis that the noise induced by fluoride and azide arises from spontaneous activity in the transduction mechanism. The phenomena described in chapters 2 and 3 parallel the recent findings of Fein and Corson (1979, 1981) and suggest that a key to the pharmacological dissection of the intermediate processes of phototransduction may lie in the need for cellular energy to quench the light-induced excitation of the system.

**The biochemical approach**

This approach involves the analysis, in vitro and in vivo of the biochemical elements within the photoreceptor that are modulated by light.

Considerable progress has been made, recently, in the biochemistry of vertebrate rod outer segments (review: Bownds, 1980). cGMP appears to be a likely candidate for the internal transmitter in rod outer segments. The concentration of cGMP is rapidly modulated by a light-activated cGMP-ase, contained in the membrane discs that also contain rhodopsin. One lesson to be learnt from the biochemistry of cGMP regulation by light is the requirement of the mechanisms that activate and inactivate several stages in the proposed chain of events for cellular energy, in the form of ATP and GTP.
For invertebrate photoreceptors, the biochemical approach has been hampered by the small quantities of material available from an insect retina or from a *Limulus* giant photoreceptor, compared with those obtainable from a frog retina. The future, however, is sure to bring more sensitive biochemical assays to overcome this difficulty.

**The genetic approach**

*Drosophila* mutants with apparent defects in the intermediate processes of phototransduction are now available (review; Pak et al, 1980). The characterization of the gene products involved promises to be a powerful tool for the future. The first requirement is that the mutants exhibit interesting deficiencies, which can be directly attributed to modifications in the proteins involved in phototransduction. However, it is not easy to record the trans-membrane electrical potentials generated by *Drosophila* photoreceptors, due to their small size. Conclusive demonstration of modifications to the light-sensitive conductance will therefore be hard to obtain, but some progress is clearly being made in this direction by Pak's group.

The discussion above should have made it clear that, at this stage in our investigations, data on the kinetics, pharmacology, biochemistry and genetics of phototransduction in as many species as possible are required to piece together the puzzle of phototransduction. Although conclusive biophysical evidence is difficult to obtain from insect photoreceptors, data gathered from them has provided a significant contribution to our understanding of phototransduction in the past, and, I hope, will continue to do so in the future.
CHAPTER ONE:

THE ELECTROPHYSIOLOGY AND ANATOMY OF PERFUSED

LOCUST RETINULA CELLS
SUMMARY

1) The anatomy and electrophysiology of a superfused preparation of the retina of a locust's compound eye are examined.

2) Photoreceptors in the preparation are electrophysiologically viable for at least 4hr during experiments involving dim or brief illumination. The maximum responses to flashes of light and the resting potentials of the photoreceptors are typical of those that can be recorded from intact eyes.

3) Three conductance pathways across the photoreceptors' membranes are demonstrated: a depolarizing conductance that mediates the peak response to a light flash; a voltage-dependent conductance that rectifies the response to depolarizing currents; and a slowly inactivating potassium current that produces a hyperpolarization after bright flashes of light.

4) The peak depolarization to a light flash and the voltage noise accompanying steady illumination are both reduced to the same extent on replacement of extracellular sodium with choline and both the noise and the peak flash response appear to share a reversal potential of about 0 mV. These observations are consistent with the idea that the noise arises from fluctuations in the light-sensitive depolarizing conductance (Dodge, Knight and Toyoda, 1968).

5) The electrical time constant of the photoreceptor is shown to be approximately 10 ms and the cable properties of the photoreceptor are estimated from its input resistance.

6) The anatomical integrity of the photoreceptors is unaffected by superfusion in darkness over a period of 2hr. The preparation is capable of synthesising an enlarged rhabdom when it is superfused over
the dusk period. Superfusion under constant intense light, comparable to sunlight, for 2hr causes vesiculation and shrinkage of the rhabdom. Thus, although superfusion _per se_ does not appear to damage the preparation, the superfused photoreceptors undergo pathological degeneration under intense light.
Figure 1.1.

The dissected eye of a locust is shown in the superfusion chamber. The photoreceptors, pigmented red, on the right-hand rim of the eye, are exposed along their entire length distal to the basement membrane. A micropipette is positioned above the photoreceptors. To the far right is the indifferent electrode. The scale bar indicates 1 mm.
INTRODUCTION

Locust photoreceptors, like those of other apposition compound eyes, cluster in groups, called ommatidia, behind each facet of the eye. Six long photoreceptors and two shorter photoreceptors (Wilson et al., 1978) each contribute some photoreceptive microvilli to the central rhabdom to form, in transverse section, a petal-like arrangement (fig. 2). Each ommatidium is approximately 300 μm long, and the axons of the photoreceptors project a further 100 μm below the basement membrane of the retina. The photoreceptors are surrounded by a matrix of glial tissue (fig. 2).

Scholes (1964) reported that photoreceptors in superfused retinal slices of a locust compound eye were capable of generating discrete events of amplitude 0.1 to 1 mV. The amplitudes of these events contrast sadly with the 1 to 10 mV 'bumps' later recorded from photoreceptors in intact eyes by Lillywhite (1977) and others. There are, of course, a large number of possible reasons for the poor quality of earlier recordings. The object of the preliminary work for the present thesis was to develop a superfused preparation which exhibited responses that were more like those of the intact eye.

An obvious first step was to minimize the mechanical damage to photoreceptors during preparation. The use of a hand-held or a vibrating razor to make parallel cuts through the retina and lamina along the ommatidial axis invariably resulted in the severing of retinula cell axons as they entered the tough, elastic basement membrane. The slice preparation was therefore abandoned in favour of one in which ommatidia close to the base of the eye were exposed along their entire length distal to the basement membrane, by the use of a single razor cut. The base of the eye and the surrounding cuticle then provided support for the photoreceptors. This chapter explores the
basic electrophysiology and anatomy of such a preparation and it constitutes a starting point not only for the present thesis, but also for those who might wish to use such a preparation in the future.
Figure 1.2

A TS through the preparation taken through the cone cells adjacent to the cornea (right) and through the distal nuclear region of the photoreceptors (left). The section extends vertically from the basement membrane at the bottom to the cut surface at the far top of the section. Notice that the 'petals' of photoreceptors are imbedded in a matrix of glia. This preparation was perfused from 22.00 hr until 1.00 hr and it therefore exhibits large night rhabdoms.
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</tbody>
</table>

All pH 7.0

High-calcium saline is used only in chapter 3
MATERIALS AND METHODS

Dissection

Adults from a laboratory culture of *Locusta migratoria*, reared under a 16 hr/8 hr cycle of light and darkness, were dissected under dim, red light. The animals were decapitated and one compound eye was removed by slicing through the cuticle around the eye. The eye was then waxed, facing upwards, upon a perspex block. A cut was made across the eye, close to its base and parallel to it, and the top section was removed to expose a ring of photoreceptors around the rim of the remaining eye section, while the connections of the photoreceptors to the lamina remained intact. Before dissection, the locust had been dark-adapted for 20 min. All the electrophysiological data was gathered during the day period.

The perspex block upon which the eye had been mounted was placed in a perspex chamber, volume 1 ml. The half-time for solution changes was approximately 20s.

Salines

Saline compositions are shown in Table 1. The salines were oxygenated in separate bottles and passed across the retina at a rate of 3-5 ml/min. A tap at the entrance of the superfusion chamber was used to switch between solutions. Fig. 1 is a photograph of the preparation during recording.

Stimulation and recording

Photoreceptors were stimulated along their exposed length with light from a green light-emitting diode (Siemens LD-57C), peak wavelength 560 nm. To saturate a cell's response to light, white light from a tungsten microscope lamp was focused onto the preparation.
During continuous, dim illumination of the preparation, quantum bump rates of 1 to 10 per second were typically recorded at a light intensity of $10^9$ photons.cm$^{-2}$.s$^{-1}$ incident on the retinal surface. Thus the light intensities quoted elsewhere in this thesis can be approximately converted to the units of effective photons per receptor per second from those of photons.cm$^{-2}$.s$^{-1}$ by dividing by $10^9$. Using this factor, steady state depolarizations of 15-20 mV are produced by light intensities of approximately $10^4$ effective photons.s$^{-1}$ per receptor [$10^{13}$ photons.cm$^{-2}$.s$^{-1}$]. These figures are consistent with the data of Howard (1981) obtained from intact eyes.

Single micropipettes of resistance 50-100 MΩ and 0 glass double micropipettes were pulled from borosilicate glass and filled with 3M potassium acetate. An amplifier similar to that used by Gage and Eisenberg (1969), with output to the bath indifferent electrode, was used to record from micropipettes impaling the distal end of photoreceptors on the cut surface of the retina. A voltage-to-current converter (Gage and Eisenberg, 1969) enabled constant current to be passed down one barrel of double electrodes irrespective of the electrode resistance. The input of the voltage to current converter was taken as a measure of the current passed. Direct measurements of currents of up to 10 nA passed through electrodes of the type used showed no distortion of the linearity of the V-I conversion. The indifferent electrode was a silver wire connected to the bath by a 3M potassium acetate/agar bridge. Fig. 3 illustrates the set-up.
Figure 1.3

The electronic recording set-up used throughout this thesis. To the left is the voltage to current converter and in the middle is the recording amplifier. The preparation and the indifferent electrode are also illustrated. The preamplifier and current-injection probe were designed by Dr. Gert Stange.
Computations on voltage noise

Records of cell voltage noise were filtered with a high-pass RC filter (half-power frequency 0.5 Hz with a slope of 6 dB per octave) and a 4-stage, active, low-pass filter (half power frequency 160 Hz and slope -48 dB per octave). The filtered record and an unfiltered one were digitized 'on line' by a PDP-8E computer at a rate of 500 samples per second and stored on floppy disc. Mean membrane potential was calculated from the unfiltered record while the filtered one was used for noise analysis.

Recording from double electrodes

While the resistive coupling between barrels was less than 500 kΩ when measured with an electrode in saline, it rose to several MΩ when the electrode first penetrated a cell. This extra coupling could be removed by withdrawing the electrode by 2-3 μm. Pressure of the electrode on the cell membrane opposite to the point of penetration would produce a resistance which might account for this coupling. To estimate the coupling when inside cells, a photoreceptor's response was saturated while the cell's resistance was measured by applying a square wave of current, amplitude 0.4 nA, down one electrode barrel. Shaw (1969), using two electrodes in the same cell, shows that the cell membrane is short—circuited when the cell's response is saturated. Any resistance using double electrodes under the same conditions is therefore attributable to coupling. For data to be considered valid, a cell had to show an input resistance under saturating illumination that was less than 10% of that in darkness (ie. <2M coupling). This condition limits the useful range of the electrodes to those membrane potentials at which the cell input resistance is significantly greater than the coupling resistance. If we set the criterion that the cell input resistance (chord resistance)
should be at least 4 times greater than the coupling resistance, then we are limited in our investigation of the voltage-current characteristics of the cell to depolarizations that are less than 30 mV from the normal resting potential in darkness.
THE ELECTRICAL PROPERTIES OF THE PHOTORECEPTOR

Introduction

The giant photoreceptors of Limulus and Balanus have been intensively investigated using the voltage clamp technique and two intracellular electrodes (Millecchia and Mauro, 1969; Brown et al., 1970). Because insect photoreceptors are smaller and harder to see, previous analyses of their electrical properties have relied largely on current injection through single electrodes and bridge circuits. These studies have the great disadvantage that the measurements of the membrane potential during the injection of current are distorted due to the non-linearity of the resistance of the recording electrode and through possible changes in its resistance as it enters a cell. Recordings using θ glass electrodes (Brown and Flaming, 1977) allow current to be passed down one electrode while only a small resistance couples it to the other barrel. Reasonably accurate potential measurements can then be obtained over the range of membrane potentials for which the cell input resistance is significantly greater than the coupling resistance.

Using these techniques, I have repeated and extended the observations of Winter (1967), Baumann (1968), Shaw (1969) and Muijser (1979) on the electrical properties of insect retinula cells. The results suggest that, as is known to be the case in Limulus and barnacle photoreceptors (Fain and Lisman, 1981; Brown et al, 1970), the potential generated by light across the membranes of locust retinula cells is produced by several light- and voltage-dependent conductances. In gathering this data, I have established simple techniques of measuring the input resistance of the cell and the effects of ionic substitutions on the conductances involved. These techniques will be used in the investigations of the more complex
phenomena described in chapters 2, 3 and 4. The results of this section provide a sketch of the electrical processes in the photoreceptor, rather than a comprehensive description.
Figure 1.4.

The relationship between injected current and the steady-state voltage change (V-I curve) for photoreceptors impaled with 0-glass electrodes. Filled circles show the relationship in darkness: open circles show the relationship when the light-induced depolarization had reached a steady-state plateau.

(a) Data from a single cell

(b) Mean data from 6 cells. Bars indicate the standard error of the mean.

The light intensity used was $3 \times 10^{12}$ photons.cm$^{-2}$.s$^{-1}$.
Results

The membrane potential in darkness

Photoreceptors could be stably penetrated with micropipettes for up to 2 hr. Experiments on a given preparation were usually complete within 1 hr of dissection. In some longer experiments, photoresponses of maximum amplitude >40 mV could be recorded 4 hr after dissection. Locust photoreceptors in a superfused eye maintain resting potentials, $V_m$, that are about 50 mV below the potential of the bathing saline. Direct jumps of potential were rarely seen upon entering a cell, possibly because of penetration through intervening glial cells. $V_m$ was therefore recorded on withdrawal of the electrode out of the retina at the end of an experiment.

The photoreceptors exhibit rectification of outward-going currents (fig. 4) (Winter, 1967). When hyperpolarized, the cell behaves as an Ohmic resistor ($R_{in} = 17 \pm 3 \text{M}\Omega$ (SE); $n = 6$) but the chord resistance drops to 9MΩ at a depolarization of 21 mV. The depolarizing voltage steps caused by current injection reach a steady-state value within the 20 ms time resolution imposed by the artifact of the electrode coupling capacitance. The rectification is therefore a rapid process and it may contribute to the attenuation of the peak response to a light flash as well as to the plateau response seen on prolonged illumination.

The response to light

The response to continuous dim light consists of a train of randomly occurring depolarizing events ('bumps') (fig. 5a). Each bump represents an effective absorption of a single photon (Lillywhite, 1977).
Figure 1.5

(a) The upper trace shows single photon events (bumps) recorded in response to illumination of intensity $10^9$ photons.cm$^{-2}$.s$^{-1}$.

The lower trace shows a recording from the same cell when in darkness.

(b) A series of responses to dim flashes of duration 1ms. The recording was a.c. coupled, hence the apparent hyperpolarizations following the responses. The arrows indicate flashes that failed to elicit any response, demonstrating the quantal nature of the response.

Light intensity; $3 \times 10^{11}$ photons.cm$^{-2}$.s$^{-1}$.

(c) The response of a photoreceptor to a flash of intensity $9 \times 10^{13}$ photons.cm$^{-2}$.s$^{-1}$. The peak depolarization, $V_p$, and the hyperpolarizing afterpotential, $V_h$, are indicated.

The arrow indicates the peak of the initial transient or 'spike'.
The response to a very dim flash consists of a random number of superimposed bumps, of different latencies (fig. 5b). Lillywhite (1977) has shown that the number of bumps produced in response to each flash of light delivered to a locust photoreceptor follows the Poisson statistics of photon capture by that receptor.

In response to a flash of high intensity, the bumps fuse to form a graded depolarization whose amplitude saturates at a potential, $V_{\text{max}}$ of $-5 \text{ mV} \pm 5 \text{ mV}$ ($n=6$). The waveform of the response to a 100ms flash, producing a peak depolarization of 60-80% $V_{\text{max}}$, has 4 distinct components (fig. 5c)

1) a rapidly rising transient (or spike?)

2) a slower rise to a peak depolarization, $V_p$

3) a falling phase

4) A hyperpolarizing afterpotential, $V_h$

Both the initial 'spike' and the afterpotential appear only in freshly penetrated cells.

Hyperpolarizing currents increase and depolarizing currents reduce the peak depolarization, $V_p$. $V_p$ is linearly related to $V_m$ within the range over which potential measurements were reliable (up to a depolarization of 25 mV) (fig. 6d). The extrapolated reversal potential of the peak response was $+1 \text{ mV}$ (regression through the mean results from 6 cells). The linear relationship between $V_p$ and $V_m$ does not imply that the cell behaves as an Ohmic resistor; clearly it does not. The apparent linearity probably arises from the large response evoked by the test flashes used for fig. 6d. The large light-induced conductance may be shunting any variations in the light-insensitive conductance pathways. Some cancellation of the
Figure 1.6

(a) Responses of a photoreceptor to flashes of duration 100ms, intensity $9 \times 10^{13}$ photons.cm$^{-2}$.s$^{-1}$ during the passage of current. The upper trace of each record shows the electrode potential, while the lower trace shows the input to the V-I converter used for generating current Same cell as fig. 4a.

(b) Light-induced noise recorded during the passage of current that depolarized the cell from -40 mV to -32 mV (+2nA) and hyperpolarized the cell to -57 mV (-2.5nA). Noise recorded before illumination, at the resting potential of -50 mV is, also shown. Same cell as fig. 6a. The light intensity used was $3 \times 10^{12}$ photons.cm$^{-2}$.s$^{-1}$.

(c) The peak response to a flash ($V_p$, triangles) and the relative r.m.s. amplitude of LIN (squares) are plotted as a function of membrane potential (same cell as figs. a and b). The lines are the linear regression.

(d) As 6(c), but the mean results from 6 cells are used, with bars indicating standard errors.
non-linearities (voltage-dependence) of the resting and light-induced conductances may also be occurring. In barnacle photoreceptors a similar, spurious linearity can be observed under current clamp (Brown et al., 1970).

In exceptional cells, the responses to light could be reversed at potentials between 0 and 20 mV (fig. 7). However, the electrode coupling resistance at these potentials is likely to be comparable to the cell input resistance, making the observed reversal potential a dubious over-estimate of the true reversal potential.

**The response to steady illumination**

Like other invertebrate photoreceptors, the initial peak response to sustained illumination is followed by adaptation to a lower ('plateau') response. Because of the discrete nature of the response to a single photon, the response to steady illumination is accompanied by voltage noise (light-induced noise, LIN) (fig. 6b) that arises from both photon shot noise and the transducer noise described by Lillywhite and Laughlin (1979).

The cell's input resistance is reduced during the steady state response to light (fig. 4). The r.m.s. amplitude of LIN, like $V_p$, is linearly related to $V_m$ during current injection (fig. 6d). Artifactual noise introduced during current injection was checked by passing current through the cells in darkness. It limits the range over which the relationship between LIN amplitude and $V_m$ can be investigated to potentials attained with currents of <3 nA. The extrapolated null potential of LIN is similar to that of the peak response to light, being +4.6 mV (n=6), which is consistent with the suggestion that LIN and the peak response to a flash of light are generated by the same conductance mechanism.
Figure 1.7

Recordings from a photoreceptor stimulated with 10ms flashes of intensity $9 \times 10^{13}$ photons.cm$^{-2}$.s$^{-1}$. The upper records, of potential, show that the response of the cell may be reversed by the injection of large amounts of depolarizing current.

Figure 1.8

(a) Recordings of responses of a photoreceptor to a flash (black bar) during current injection. Note the reversal of the hyperpolarizing afterpotential during the passage of hyperpolarizing currents. The cell was stimulated by light of intensity $9 \times 10^{13}$ photons.cm$^{-2}$.s$^{-1}$.

(b) Same cell as fig. 8a. A plot of the amplitude of the potential, $V_h$, recorded 400 ms after the onset of the stimulating flash, as a function of the resting potential of the cell during current injection. Note the reversal of $V_h$ when the cell is hyperpolarized.

(c) Similar data to those of fig. 8b recorded from another cell.
The hyperpolarizing afterpotential

Most freshly penetrated cells showed a slowly recovering, hyperpolarizing afterpotential, $V_h$, after the response to a bright flash. This hyperpolarization could be reversed at potentials that were 5-15 mV below the resting potential (fig. 8). It therefore constitutes a different conductance from that generating the peak response to a flash.

The principle ion that might carry a current, so as to generate a hyperpolarization, is potassium. Many neurons maintain a resting potential that is a few mV above the equilibrium potential, $E_k$, for potassium ion movements. It was found that increasing $E_k$ by removing external potassium caused $V_h$ to increase, while reducing $E_k$ by increasing the external potassium concentration to 20 mM caused $V_h$ to reverse, as the photoreceptor depolarized by up to 20 mV (fig. 9). The time course of the hyperpolarizing response, as well as the eventual decline of $V_h$ when the membrane potential is increased to more than -35 mV by current injection, has much in common with the observations of Hanani and Shaw (1976) on barnacle photoreceptors. They explained the biphasic effect on $V_h$ as indicating that a Ca-dependent $K^+$ current was involved, so that large depolarizations reduced the inward calcium current that activated the potassium current. In the locust preparation it is not possible to examine the effects of external calcium on $V_h$ in isolation. This is because an increase in the external calcium concentration causes a shortening in duration of the depolarizing component of the response (chapter 3). In many cells, $V_h$ is a balance between hyperpolarizing and depolarizing afterpotentials, so that any reduction in the time course of the latter may cause an increase in $V_h$ without changing the potassium current.
Figure 1.9

Upper: superfusion with potassium-free saline (0 K⁺) results in an increase in the hyperpolarizing afterpotential, while subsequent superfusion with high-potassium saline (20mM K⁺) results in a depolarization of the cell and a reversal of the afterpotential.

Lower: the response to each flash is shown in more detail. The flash intensity used was 9 x 10¹³ photons.cm⁻².s⁻¹.
(a) The potential recorded during a 0.5 nA current step through a glass electrode impaling a cell.

(b) The potential recorded through the same electrode as was used in fig. 10(a) but with the electrode withdrawn from the cell.

(c) The potential traces of fig. 10(a) and (b) were digitized and aligned so that the current pulse in each case began at the same sample. The potential recorded in 10(b) was then subtracted from that in 10(a) and the result plotted. The resulting trace approximates to the response of the cell's potential to a step change in the injected current.

(d) & (e) Traces from other cells calculated similarly to 10(c).

All vertical scale bars indicate 5 mV.
The time constant of the cell

Capacitive coupling between the barrels of the θ glass electrodes produced large artifacts when the electrode was in saline (fig. 10b). These artifacts prevented a direct measurement of the cell's time constant. Only the tail of the response-transient to a current step could be resolved (fig. 10a). Subtraction of the records obtained outside cells from those obtained inside cells gave a response to a small current step that could approximate to the true transient. This treatment assumes that the capacitive and resistive coupling between electrode barrels did not change when a cell was impaled. This assumption may seem arbitrary, but it could be roughly checked by observing transients to current steps applied when the cell was saturated by light, so that \( R_{\text{in}} \) and the membrane time-constant, \( T \), are greatly reduced. (see Methods).

The potential during the response to a small hyperpolarizing current step declines to 1/e of its initial value within 10-15ms. This figure represents the longest time-constants of the cells penetrated, because only those recordings in which the tail of the transient were clearly separable from the electrode artifact were analysed in detail.

Sodium replacement

Complete replacement of the sodium in the perfusate by choline reversibly reduces the response to a brief, weak flash of light from 14 mV ± 1 mV (SE) to 2.7 mV ± 0.4 mV (SE) within 150s (fig. 11iii). The response could not be completely abolished, as found by other investigators of superfused bee, crayfish and Limulus compound eyes (Fulpius & Baumann, 1969; Stieve, 1964; Wulff, 1973). These authors have suggested that the failure to completely abolish the reponse might be due both to a buffering of the sodium concentration in the
Figure 1.11

(i) The effect on the response to a flash of total replacement of superfusate sodium by choline. The recordings were made (left to right) in sodium saline; 2 mins after switching to choline saline and 2 mins after reverting to superfusion with sodium saline. Receptor potentials were elicited by light flashes at 30s intervals throughout the experiment.

Flash intensity; $10^{13}$ photons.cm$^{-2}$.s$^{-1}$; flash duration 10ms.

(ii) Effects of total replacement of superfusate sodium by choline on LIN and the steady response evoked by continuous illumination. The recordings were made: (a) Before illumination; (b) During illumination when in sodium saline; (c) 2 mins after switching to choline saline; (d) 4 mins after reverting to sodium saline while illumination continued; (e) 1 min after the cessation of the illumination. The vertical displacement of the traces indicates their relative d.c. potential. The light intensity was $3 \times 10^{12}$ photons.cm$^{-2}$.s$^{-1}$.

(iii) The mean response amplitudes, $V_p$, and resting potentials, $V_m$, of 6 photoreceptors during sodium replacement experiments as described for (i) above. Bars indicate standard errors of the means.

(iv) The relative r.m.s. LIN (solid line) and mean light-induced depolarization (dashed line) of 6 cells during the experiment described above in (ii). Bars indicate standard error of the mean.
extracellular space around the photoreceptors and due to a lack of selectivity for sodium by the light-activated conductance. On removal of the choline saline, a slight depolarization of the cell developed.

As might be expected from the effect of sodium replacement on the response to flashes, the r.m.s. amplitude of LIN and the light-induced depolarization of a continuously illuminated cell are both reduced in choline saline. The depolarization of 6 cells fell from 8.5 mV ± 1 mV to 4.7 mV ± 1 mV, while the amplitude of the LIN dropped to 22% of its original value (fig. 11 iv). During the introduction of choline saline, the drop in LIN amplitude closely followed the drop in the depolarization. However, on the replacement of sodium, the cell depolarized faster than the LIN recovered. This rapid depolarization may be related to the depolarization that developed in darkness immediately following the re-introduction of sodium saline.
DISCUSSION

Many of the results of this chapter confirm the previous studies of Stieve (1964), Winter (1967), Fulpius and Baumann (1969), Wulff (1973) and Muijser (1979) who have investigated photoreceptors in the compound eyes of crayfish, locust, bee, Limulus and fly. In the locust retinula cell this chapter demonstrates three possible conductance mechanisms that contribute to the cell's response to light.

1) A rapidly activating and rapidly inactivating conductance with an apparent reversal potential of about 0 mV. This is responsible for the peak transient response to a flash of light and for LIN. This conductance certainly produces an increased permeability to sodium ions, and an increase in the permeability to other ions cannot be excluded.

2) Antagonistic to this depolarizing conductance there exists a rapidly activating voltage-dependent conductance which rectifies the response to depolarizing currents.

3) A slowly inactivating conductance that is responsible for the hyperpolarization that follows the response to bright flashes. This conductance has a reversal potential that is 5 to 15 mV below a cell's resting potential.

Conductance (3) will contribute to the fall in potential from the peak transient to the plateau phase of the response to a step of bright light. Tsukahara (1980) has shown that the reversal potential of the plateau response of Locusta retinula cells is lower than that of the peak transient response. A delayed potassium conductance, such as conductance (3), would account for this result.
The use of voltage- or calcium-dependent conductances to antagonise the depolarizing action of the light-activated sodium conductance is an apparently ubiquitous stratagem to aid adaptation in invertebrate photoreceptors. Pepose and Lisman (1978) have argued that these conductances serve to increase the dynamic range of the photoreceptor by hyperpolarizing a strongly illuminated photoreceptor back into the potential range in which the response to a flash is log-linear. This hyperpolarization complements the decrease in the sensitivity of the depolarizing conductance to light which must also occur (Fein and Charlton, 1977; Tsukahara and Horridge, 1977). The drawback of opening antagonistic potassium channels is an increased rate of Na\(^+\)/K\(^+\) exchange across the cell's membranes. This is particularly important for small cells. Coles and Tsacopoulos (1979) report that K\(^+\) loss from bee retinula cells during repetitive stimulation is substantial. Presumably this loss of K\(^+\) is eventually counteracted by the action of ion pumps.

**The effect of rectification on the response-intensity curve**

The rapidly activating, rectifying conductance may reduce the peak response to a flash of light. Its effect may be calculated by the following argument.

Consider the cell as being represented by the following circuit:

\[\text{The circuit includes only the voltage-dependent conductance, } R_D, \text{ and the light-activated conductance, } R_L.\]
Inside potential = \( V \)

\[ I_C = 0 \text{ in steady state} \]

\[ I_D \]

\[ R_D = f(V) \]

\[ R_L = f(h\omega) \]

\[ V_{\text{rest}} = -50 \text{ mV} \]

Outside potential = 0

In darkness, when \( R_L = \infty \), \( I_D = -f(V - V_{\text{rest}}) \), where \( V_{\text{rest}} \) is the resting potential of the cell in darkness. From the V-I curves obtained in darkness (fig. 4a), a polynomial expression for \( I_D \) can be calculated.

We also have, during illumination, under open circuit

\[ I_D = -I_L = \frac{V}{R_L} \quad (1) \]

and so

\[ R_L = -V/f(V-V_{\text{rest}}) \quad (2) \]

If \( R_D \) is constant, then;

\[ I_D = -(V-V_{\text{rest}})/R_D \quad (3) \]

and, using Eq. 1 to substitute for \( I_D \),

\[ R_L/R_D = -V/(V-V_{\text{rest}}) \quad (4) \]

-which is the 'self-shunting' relation (Martin, 1955)
Figure 1.12

(a) The relationship between the peak depolarizing response of a photoreceptor, $V_p$, and the magnitude of the light-induced conductance, $G_L$. The dashed line shows the response expected for a linear, self-shunting membrane, while the solid line is the relationship expected if rectification occurs as in fig. 4a.

(b) The data of Lillywhite and Laughlin (1979). The response of a photoreceptor is plotted as a function of the effective light intensity per stimulating flash. Dashes, as in fig. 12(a) show the prediction of the self-shunting model, while dots describe the response predicted after further correction for the dispersion of bump latencies. The solid line is the experimentally determined curve. It is clear that a further correction for rectification as is shown in (a) will not flatten the dotted curve sufficiently to fit the extremely flat experimental curve above depolarizations of 10 mV.
12a  

\[ V_p \quad mV \]

G \_L \_M \Omega^{-1}

12b  

Response amplitude (mV)

Effective photons \( (N_C) \)

0  25  50  75
To calculate $R_L$ from Eq. 2, the V-I curve of fig. 4a was used. The curve was approximated by a 4th. order polynomial. For each value of $V$, a value of $I_D$ is obtained. $R_L$ is then calculated from (2).

Each photon is now assumed to contribute an equal amount to the light-activated conductance, $G_L = 1/R_L$. Thus a plot of $G_L$ against $V$ (Fig. 12a) can be used to compare the response-intensity relation expected from a self-shunting model (Eq. 4) with that expected if rectification is also considered. As can be seen in fig. 12a, the rectification increases the light intensity (conductance increase) required to elicit a response of more than 5 mV. However, comparison with the data of Lillywhite and Laughlin (1979) (Fig. 12b) shows that the actual response-intensity relation is far flatter at depolarizations of more than 10 mV than rectification can account for, even if we also take their correction for the flattening caused by the scatter in bump latencies. The explanation, offered by Laughlin and Lillywhite (in preparation), that the size of the conductance increase per photon decreases at high light intensities seems a likely explanation for the discrepancy.

The cable properties of the retinula cell body

A calculation of the cable properties of the cell enables an estimate to be made of the decrement of visual signals as they travel down the photoreceptor towards the brain. The cable decrement is also important if we wish to evaluate the reliability of the current-clamp data described above. Conductance changes are activated by light along the whole length of the cell and we therefore wish to uniformly clamp the cell's potential by current injection at the point at which the electrode penetrates it. This will not occur if cable decrement is too
<table>
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<td>Mean microvillar length</td>
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<td>Total cell surface area per μm length</td>
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<tr>
<td>Cross sectional area of cell</td>
<td>34 μm²</td>
</tr>
<tr>
<td>Length of cell (cornea to basement membrane)</td>
<td>300 μm</td>
</tr>
</tbody>
</table>
high. Estimates of the capacitance of the cell from its time constant also enable us to check estimates of the membrane surface area that are obtained anatomically.

Using 9 glass electrodes, a value of $17\Omega - 3\Omega \text{ (SE)}$ was obtained in this chapter for the input resistance of locust photoreceptor. This value is higher than that previously reported by Shaw (1969) [$9.4\Omega - 1\Omega \text{ (SE)}$] and it calls for a re-calculation of the estimated cable length-constant and the membrane resistivity using the formulae of Weidmann (1952). The analysis that follows makes the following assumptions:

1) We neglect the axon and regard the cell as a short cable terminated at both ends by an open circuit.

2) We neglect the effect of the weak electrical coupling that may occur between retinula cells (Lillywhite, 1978).

3) We include the microvillar surface area in the total surface area of the cell.

The dimensions used for the cell were measured from electron micrographs of TS's of the superfused eye and from the data of Wilson et al (1978). They are given in Table 2.

From Weidmann (1952) we have: 

$$R_{in} = \frac{r_i \cdot \lambda \cdot \coth (L/\lambda)}{}$$

where $r_i$ is the intracellular resistance per cm of cell, equal to the internal resistivity, $R_i$, divided by the cross-sectional area of the cell; $\lambda$ is the cell length constant; $L$ is the length of the cell body (300 $\mu$m); and $R_{in}$ is the input resistance of the cell ($17\Omega$).
Assuming a value of 100 $\Omega \cdot \text{cm}$ for $R_i$, Eq. 5 yields a length constant of 390 $\mu\text{m}$ or $4/3 \, \text{L}$. From $\lambda$ and $r_i$, we can calculate $R_m$, the resistivity of the plasma membrane.

$$R_m = \lambda^2 r_i a_p$$

where $a_p$ is the membrane area per unit length of cell.

Given $\lambda = 390 \, \mu\text{m}$, $R_m$ is $2.6 \, \text{k}\Omega \cdot \text{cm}^2$.

We can also calculate the reduction in the amplitude, $V_L$, of a steady signal recorded at one end of the dark-adapted cell relative to its amplitude, $V_o$, at the other end, where it is initiated,

$$\frac{V_L}{V_o} = \frac{1}{\cosh \left( \frac{\text{L}}{\lambda} \right)}$$

which yields a value of 75% for $V_L/V_o$. This estimate is consistent with the negligible contribution of the scattering of photon hits along the rhabdom to the variance in bump amplitude (Lillywhite, 1978). Even if the conductance change is localized to the site of a photon-hit, very little attenuation of the response will be recorded by an electrode in the centre of the cell.

When estimating the attenuation of visual signals in daylight, it must be remembered that the membrane resistance of a cell depolarized to about 10 mV by light (fig. 4) is about halved. The new value for $V_L/V_o$ is 64%.

Given $r_m$, the membrane resistance per unit length of cell, and the time-constant of the cell (see page 27), we can calculate a value for $c_m$, the membrane capacity per unit length.

$$r_m = \lambda^2 r_i = 4.4 \times 10^5 \, \Omega \cdot \text{cm}$$

$$c_m = \frac{T}{r_m} = 2.3 \times 10^{-8} \, \text{F} \cdot \text{cm}^{-1}$$
From a knowledge of the total membrane area (Table 2), the membrane capacitance per unit area of membrane, $C_m$, can be calculated to be $3.8 \, \mu F/cm^2$. This estimate appears to be reasonable when compared with the commonly accepted value of $1 \, \mu F/cm$. The measured values of $T$ and $R_{in}$ are therefore consistent with the geometry of the cell. The time constants and input resistances reported in this chapter are also similar to those obtained recently from the R 1-6 retinula cells of the fly compound eye by Muijser (1979) who found $T = 5 - 10 ms$ and $R_{in} = 14 - 20 M\Omega$. 
THE SYNTHESIS AND BREAKDOWN OF THE RHABDOM AT DUSK AND DAWN

Introduction

Like the photoreceptors of many arthropods (review; Blest 1980), photoreceptors in the locust compound eye are able to grow a larger rhabdom at dusk, which they subsequently degrade in the morning (Horridge, Duniec and Marcelja, 1981). It was therefore of interest to see whether the photoreceptors of a superfused locust compound eye were in sufficiently good condition to perform similarly. Preparations were superfused for 2-3 hr at various times of the day, either in darkness or when intensely illuminated. The locusts were maintained on a constant daily light cycle throughout these experiments with light on from 6.00 hr to 22.00 hr. We found that the photoreceptors were capable of enlarging their rhabdoms at dusk, in darkness. However the preparation could not be maintained in good anatomical condition under the intense illumination typical of daylight. The breakdown process could not, therefore, be studied under natural conditions of illumination of the superfused preparation.
Figure 1.13

(a) a TS of an Ommatidium taken at the distal nuclear region, approximately 100 µm below the cornea. The eye was dissected at 9.00 hr and superfused in darkness until 11.45 hr before being fixed in darkness.

(b) shows the rhabdom in greater detail.

The scale bars indicate 10 µm and 0.5 µm in (a) and (b) respectively.

This tissue and all the other tissue in Figs. 14&15 was fixed in the following manner: 1) Fixation in 2.5% gluteraldehyde, 0.1M Na cacodylate buffer and 0.12M sucrose. 2) Post-fixation with 1% osmium tetroxide before dehydration and embedding. The fixation was carried out by R. P. and the tissue was embedded and cut by Ms. Jadwiga Duniec.
Results

1) Perfusion of the preparation in darkness

1.1 Perfusion during the day period

Light-adapted preparations were dissected and superfused in darkness between 9.00 hr and 11.45 hr and then fixed in darkness. Transverse sections of these preparations taken at the distal nuclear layer were then compared to similar preparations of un-perfused tissue fixed at 9.00 hr from the other eye of the same animal.

The photoreceptors of the superfused eye (fig. 13) appear to be slightly swollen when compared to those of intact eyes. Rhabdom, nuclei, mitochondria and endoplasmic reticulum appeared to be unaffected by superfusion in darkness in the morning. No synthesis of a large night rhabdom occurred. Glial processes around the photoreceptors also appear to be normal in the preparation superfused in darkness.

1.2 Perfusion in darkness at dusk

Preparations were superfused between 22.00 hr and 22.15 hr in room light and then between 22.15 hr and 1.00 hr in darkness. During this period the rhabdom of the photoreceptors enlarged considerably (fig. 14). The superfused cells again appeared to be slightly swollen and lacked the prominent palisade of photoreceptors from intact animals fixed at 1.00 hr (fig. 14e). The increase in rhabdom size was, however, similar to that observed in the intact eye.
2) Perfusion under constant, intense light

Preparations were superfused under constant, intense illumination from a daylight fluorescent screen delivering $3 \times 10^{14}$ photons.cm$^{-2}$.s$^{-1}$ between 400 and 600 nm at the surface of the superfusion chamber.

2.1 Perfusion during the day period

In contrast to superfusion in darkness, superfusion in constant bright light during the day period, between 19.45 hr and 22.00 hr, had a clear adverse effect on the photoreceptors (fig. 15). Both retinula cells and glia appear shrunken when compared with photoreceptors from intact animals fixed at 22.00 hr (fig. 14a). More importantly, rhabdom membrane has been lost and the microvilli are very much shorter. Double membrane vesicles cluster around the base of the shortened microvilli and membrane distentions are formed from the neighbouring microvillar walls (fig. 15b). Double membrane vesicles also appear to be forming from the fusion of the plasma membranes of neighbouring cells (fig. 15c).

The day rhabdom of intact eyes was quite stable under the intensity of illumination used for these experiments. The breakdown of the rhabdoms of superfused photoreceptors is therefore pathological.

2.2 Perfusion over the dawn period

Preparations were dissected under a red light and superfused between 5.00 hr and 6.00 hr in darkness and then from 6.00 hr to 7.45 hr in light. Preparations fixed at 6.00 hr showed no breakdown of the large night rhabdom. Preparations fixed at 7.45 hr (fig. 15d) had rhabdoms that were smaller and more disorganized than the rhabdoms from the photoreceptors of intact animals given the same light regime.
Figure 1.15

(a) An ommatidial TS in an eye that had been superfused in constant bright between 19.45 hr and 22.00 hr before fixation.

(b) is an enlargement of the rhabdom showing (arrows) vesciculation of the rhabdom and the plasma membranes.

(c) A rhabdom from another cell which was superfused under the same conditions as in (b). Clear vesciculation of the plasma membrane may be seen (arrows).

(d) Ommatidial TS from an eye that had been superfused from 5.00 hr to 6.00 hr in darkness and then from 6.00 hr to 7.45 hr under constant bright light. As with the rhabdom of (b), shrinkage and vesciculation (arrows) of the microvilli can be observed.

The scale bars in (a) indicates 10 µm, while those in (b) and (c) indicate 1 µm.
and fixed at 7.45 hr. The rhabdoms of the latter were no different from those seen from day-state animals, so that breakdown of the night rhabdom in the photoreceptors of intact eyes must have been complete by 7.45 hr.

The distention of the bases of the microvilli in the superfused photoreceptors and also the presence of double-membrane vesicles suggested that the same destructive process was operating as had occurred in photoreceptors that were superfused in bright light during the day period. Thus while the breakdown of the night rhabdom undoubtedly occurs when the superfused preparation is illuminated at dawn it is likely that this breakdown reflects the pathological pattern described in section 2.1 as well as, or instead of, normal breakdown which is thought to be brought about by ordered pinocytosis at the base of the microvilli (Williams, in preparation).

Discussion

The anatomy and the cellular function of the photoreceptors are unaffected by superfusion in darkness at any time of day, except for the disappearance of the palisade. The superfusion chamber per se does not therefore appear to be detrimental to the cell. Superfusion in constant bright light, however results in the shrinkage of fixed photoreceptors and glia, together with the breakdown and vesiculation of both rhabdomal and plasma membrane.

Constant bright light must stress the metabolism of the receptors. Ion pumping, to maintain trans-membrane concentration gradients, and possibly transduction itself (chapter 2; Fein and Corson, 1981) require the consumption of energy in the illuminated cell. It is possible that if the perfused, strongly illuminated photoreceptor cannot maintain a sufficient supply of energy over the
two hour period, then the resulting cell injury causes the vesiculation of membrane (cf. Hoerl and Scott, 1978). The fact that both the rhabdom membrane and the plasma membrane are seen to vesiculate favours this hypothesis over one involving a more specific interaction between the microvillar membrane and light. The disordered vesiculation seen is similar to that described by Blest and Day (1978) when spider eyes are brightly illuminated at night. As Blest (1980) points out, the collapse of the rhabdom seen in such pathological states is quite different from the ordered pinocytosis that occurs in the photoreceptors of many species over a natural dawn.

The preparation clearly has a potential for the investigation of the biochemical control of rhabdom synthesis at dusk. While the preparation is perfused, the effects of drugs on the process can be evaluated. A preliminary study on protein synthesis carried out, following the anatomical investigation described above, has shown that preparations superfused between 22.00 hr and 1.00 hr in the presence of 40 μM cyclohexamide are still capable of generating a fully sized night rhabdom. Protein synthesis over the dusk period may not, therefore, be necessary for rhabdom growth - instead a store of material may be built up through the day and utilized at dusk, as is suggested by the progressive increase in the ability of locust photoreceptors during the afternoon and evening to synthesize a fully enlarged night rhabdom (Williams, in preparation; also Naessel and Waterman (1979) and Stowe (1981) in crabs).

Biochemical investigations into the breakdown process will have to overcome the spontaneous rhabdomal breakdown that occurs when the preparation is strongly illuminated even during the day period. The intensity of light used is about 100 times that required to fully light adapt the electrophysiological response of the cells to light.
Future experimenters might, therefore, try to use less light to induce breakdown and so avoid the pathological effects of intense light. The exposure to light used in these experiments was far more intense and prolonged than any used in the electrophysiological experiments described in this thesis. The maximum intensity applied continuously when studying the electrophysiology was $3 \times 10^{12}$ photons.cm$^{-2}$.s$^{-1}$, and then it was only applied for ten minutes while studying the effects of sodium replacement. This may explain why no gross deterioration of the response of the photoreceptors was recorded during the two hour-long electrophysiological experiments.
CHAPTER TWO:

SUPPRESSION OF NOISE IN A PHOTORECEPTOR BY OXIDATIVE METABOLISM
Suppression of Noise in a Photoreceptor by Oxidative Metabolism

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Summary. 1. Intracellular recordings were made, using glass micropipettes, from retinula cells in a superfused, isolated locust compound eye. Spontaneous voltage noise, generated across the photoreceptors' membranes, increases when a cell's metabolism is suppressed by anoxia or by superfusion with saline containing 1 mM azide.

2. The increase in the voltage noise develops from a noisy afterpotential, which follows each response to light flashes delivered during treatment with azide. The noise may reach a peak-to-peak amplitude of 2 mV in severely affected cells.

3. Upon removal of azide, or re-oxygenation, the increased noise level and the depolarization are abolished within 15 min.

4. The noise is shown to arise from the summation of depolarizing shot events of amplitude 40 μV.

5. It is proposed that the voltage noise results from the residual random activation of sodium channels that were open during the response to light.

Introduction

Maintenance of the resting potential of arthropod photoreceptors is dependent on oxidative metabolism (Baumann and Mauro 1973; Wong et al. 1976). A breakdown of the active transport of potassium from the extracellular space into surrounding tissues has been suggested as an explanation (Baumann and Mauro 1973). Depolarization of photoreceptors would result from a consequent reduction in the potassium gradient across the photoreceptive membrane. Baumann and Mauro also reported that the depolarization was initiated by stimulation of the hypoxic cell, the receptor being insensitive to hypoxia in darkness. This is to be expected if, as Coles and Tsacopoulos (1979) demonstrated, there is a substantial loss of potassium from stimulated photoreceptors into the extracellular space. Oxidative metabolism may therefore maintain the buffering of extracellular potassium following illumination.

This paper investigates the response of the superfused locust retina to anoxia and suggests alternative causes of the depolarization of the stimulated, hypoxic photoreceptor. Stimulation of photoreceptors in hypoxic saline or when poisoned by 1 mM azide results in a sustained depolarization following each response, accompanied by an increase in the electrical conductance of the cell and an increase in recorded voltage noise. Locust photoreceptors exhibit extremely low voltage noise levels in darkness and this study indicates that the oxidative metabolism of the cell maintains these low noise levels following stimulation. It is proposed that the afterpotential and noise result from a residual increase in sodium conductance following the receptor potential.

Methods

Recording Techniques. Details of the preparation and the recording techniques are published elsewhere (Payne 1980).

Adults from a culture of *Locusta migratoria* were dissected during the day. After decapitation, the eyes were cut open so as to expose photoreceptors on the surface of the retina along their entire length distal to the basement membrane. The cut eye and surrounding cuticle was then waxed, facing upwards, into a chamber of volume 1 ml. The eye was superfused at a rate of 3-4 ml/min with oxygenated saline of composition (mmol): Na, 200; Cl, 204; K, 3; Ca, 0.5; glucose, 90; TRIS, 10; pH 7.0.

Electrical recordings from photoreceptors were made by impaling them at their distal ends with glass microelectrodes of resistance 50-100 MΩ, filled with 3 mol potassium acetate. In some experiments, double barrelled electrodes (Werblin 1975) were used to allow current injection into a cell, while recording electrical potential down the other barrel. The electrical coupling between barrels when the electrode had impaled a cell was checked by shunting the cell's resistance using bright light (Payne 1980), and data were collected when the apparent coupling resistance did not exceed 10% of that cell's input resistance in the dark. To pass current
down the second barrel, a voltage to current converter was used (Gage and Eisenberg 1969).

The photoreceptors were stimulated along their exposed length with a green light-emitting diode (Siemens LD57C), emitting light of peak wavelength 560 nm.

**Computation and Display of Results.** The output of the microelectrode amplifier was filtered with a high-pass filter (half-power frequency, 0.5 Hz; slope, 6 dB/octave) and an active low-pass filter (half-power frequency 200 Hz; slope, −48 dB/octave). Both the filtered record and an unfiltered record (at lower gain) were displayed on an oscilloscope for photography. The vertical outputs of the amplifiers driving the two oscilloscope beams were connected to a PDP8-E computer which digitized 2 s sections of the photoresponse to each light flash (Figs. 1 and 2). This depolarization was maintained, or increased, until the azide was washed out of the bathing saline. After the removal of the azide, the cell’s resting potential and the amplitude of the voltage noise recovered completely over the next 5–15 min. A train of light flashes of the same intensity delivered before treatment with azide, or after recovery, failed to elicit a sustained depolarization or an increase in the level of voltage noise.

Cells kept in darkness during treatment with 1 mmol azide for periods of more than 5 min slowly depolarized and became noisy over the following 10 min. Stimulation of poisoned photoreceptors therefore greatly accelerates a gradual decline in membrane potential and an accompanying increase in voltage noise.

**Results**

1. Hypoxia and Azide Depolarize Stimulated Photoreceptors

During the first set of experiments, photoreceptors were stimulated every ten seconds by a brief (10–100 ms) flash of light. This produced a depolarizing response of amplitude 25–35 mV, roughly 60% of the maximum transient response, $V_{\text{max}}$, obtainable from a cell. Upon introducing saline saturated with nitrogen, or containing 1 mmol sodium azide, these cells rapidly depolarized and the amplitude of the voltage noise recorded between light flashes increased. Depolarizations of between 10 mV and 20 mV over a period of 3 min were typical. Replacement of oxygenated saline reversed the noise increase and restored the membrane potential within 2–5 min, while the complete reversal of the same effects induced by azide took 5–15 min. The effects of azide were more quantitatively consistent from cell to cell in their time course and magnitude than those of anoxia, but there was no qualitative difference in the effects of the two treatments. Azide was therefore used in most of the following experiments. Wong et al. (1979) also report that azide mimics the effects of anoxia.

If a cell was fully dark-adapted and exposed to 1 mmol azide in darkness, little depolarization or noise increase was noted over the first 3–5 min of treatment. Light flashes presented subsequently resulted in a sustained, noisy depolarization which developed from a noisy ‘tail’ in the response to each light flash (Figs. 1 and 2). This depolarization was maintained, or increased, until the azide was washed out of the bathing saline. After the removal of the azide, the cell’s resting potential and the amplitude of the voltage noise recovered completely over the next 5–15 min. A train of light flashes of the same intensity delivered before treatment with azide, or after recovery, failed to elicit a sustained depolarization or an increase in the level of voltage noise.

2. Quantitative Analysis of Voltage Noise

Voltage noise recorded from 8 dark-adapted photoreceptors in darkness had an r.m.s. amplitude of 83 µV ± 10 µV (S.E.). This included electrode and amplifier noise recorded before impalement of the cell, having a typical r.m.s. amplitude of 50 µV. Stimulation of these cells by light flashes, following treatment for 3–5 min with 1 mmol azide, resulted in an increase in the amplitude of the voltage noise recorded in darkness after the flashes to 320 µV ± 40 µV (S.E.). The most severely affected cells exhibited peak-to-peak voltage fluctuations of amplitude 2 mV superimposed upon a depolarization of 8–15 mV.

The relationship between voltage noise variance and depolarization was investigated during the recovery of these cells, as the azide was washed out of the bathing medium. The linearity of the relationship between depolarizations below 5 mV (Fig. 3, circles) and the flat maximum at 7–15 mV suggested comparison with Katz and Miledi’s (1972) analysis of shot noise due to the random opening of depolarizing ionic channels. Katz and Miledi’s theory predicts that the variance of the voltage noise, $\bar{E}^2$, and the depolarizations, $V$, will be related by:

$$\bar{E}^2 = V k a \left[ \frac{V_p - V}{V_p} \right]^3$$

where $V_p$ is the driving e.m.f. behind ion movements in the ionic pathways, $a$ is the amplitude of a voltage event due to one pathway, or channel, opening and $k$ is a constant dependent on the shape of the event.
Fig. 1. Recordings of photoreceptor responses to flashes of light delivered before treatment with azide; 3 min after treatment in darkness with saline containing 1 mmol azide; and 15 min after returning to perfusion with azide-free saline. Each record shows a high gain, a.c. coupled trace; a low gain d.c. coupled trace; and, at the bottom of the record, the stimulus. Each row displays 5 records, made before the light flashes began; during the 1st, 5th and 10th flash; and 20 s after the 10th flash. The flashes were delivered at 10 s intervals and were of duration 20 ms; wavelength 560 nm.

Fig. 2. Responses of a photoreceptor to flashes of light delivered to a cell before azide treatment (control) and following 3 min treatment with 1 mmol azide. Only the base of each response is shown, so as to emphasize the noisy afterpotential that develops following the flashes delivered during perfusion with azide. The peak amplitude of the responses was approximately 60 mV. Flash intensity 10^14 photons cm⁻² s⁻¹, wavelength 560 nm.

To apply this analysis, \( V_D \) was estimated for each cell from the depolarization at which noise was maximal, since differentiation of Eq. (1) yields a maximum at \( V_D/4 \). The variance at each depolarization was then multiplied by \( [V_D/(V_D - V)]^3 \) and the result plotted against V (Fig. 3, triangles). Straight line fits, as predicted by Eq. (1), were obtained for the 8 cells investigated. \( V_D \) was estimated at 47 mV ± 5 mV (S.E.) and, using \( k = e/4 \) (an exponential event shape), an event amplitude of 36 µV ± 8 µV (S.E.) was predicted. Given that the cell input resistance is about 15 MΩ (Shaw 1969) we can make an estimate of 40 pS for the amplitude of the conductance event underlying the voltage event using the self-shunting model of the cell from which Eq. (1) is derived. We must stress, however, that for events that are brief compared to the membrane time constant, the self-shunting model overestimates the correction required for non-linear summation of voltage events (Stevens 1976). The value of 40 pS therefore represents a rough upper limit to the true event amplitude. Even so, it is within the range of conductance expected of single ionic channels, 3–200 pS (Neher and Stevens 1977). The voltage noise and depolarization could therefore arise...
Fig. 3. Graph on the left: relationship between the increase in voltage noise variance above its final value and the depolarization recorded from the cell of Fig. 2 during the 15 min that it took to fully recover from treatment with azide and light. Circles raw data; triangles data after correction for non-linear summation (see text). Linear regression through the triangles also shown. $V_D$ (as defined in the text) was set at 40 mV and 2 s samples of noise were used for the calculations. To the right of the graph are samples of the noise recorded from another cell at the depolarizations shown. The cell was depolarized to 20 mV before wash-out of azide began. The samples were taken 290 s (12 mV), 440 s (2 mV) and 480 s (0.5 mV) after beginning to wash azide out of the perfusion chamber.

3. Power Spectral Analysis of Voltage Noise

Power spectra of the voltage noise recorded during recovery from treatment with 1 mmol azide were calculated from 10 s segments of the data (see Methods). Following the complete recovery of the voltage noise level and the membrane potential, the cell was depolarized with steady illumination, and the power spectrum of the photon shot noise (Dodge et al. 1968) was calculated so as to compare the shot noise induced by summed quantal responses to light, with noise recorded from a poisoned cell depolarized to the same extent.

In darkness, the noise recorded from a healthy cell declines as $1/f$, between 5 Hz and 180 Hz (Payne 1980). The difference spectra attributed to light-induced noise or to poisoning were calculated by taking the difference between the $1/f$ noise recorded after recovery from azide, and noise recorded during steady illumination (photon shot noise) or in the poisoned state.

The difference power spectrum of the noise recorded following stimulation of a poisoned photoreceptor is flat up to 15 Hz, declining as $1/f^2$ to $1/f^3$ at frequencies above 50 Hz (Fig. 4). As the cell recovers over a period of several minutes and the depolarization declines, the spectra of the noise are shifted to lower frequencies. This behaviour is to be expected if the power spectrum of the noise reflects the frequency characteristics of the electrical filter that is represented by the cell membrane. The time constant of this filter will increase as the input resistance of the cell increases during recovery from azide treatment (see Sect. 4), so reducing the high frequency components of the voltage noise recorded.

When compared at small depolarizations (light intensities of less than $10^{11}$ photons·cm$^{-2}$·s$^{-1}$), the noise induced by light is clearly slower and of greater variance than that seen during poisoning (Fig. 4). This is a reflection of the difference in amplitude between the shot event size of 36 pV and photon event (bump) sizes recorded from dark adapted cells of between 1 and 5 mV. As the intensity of illumination is increased to more than $10^{13}$ photons·cm$^{-2}$·s$^{-1}$ the light-induced noise and afterpotential noise become similar in both power spectral shape and amplitude. Noise recorded from a poisoned cell therefore ‘mimics’ the photon shot-noise recorded from a light-adapted cell depolarized to the same extent by strong illumination. In this respect, and in respect of the size of the shot event, the noise recorded in the afterpotential following illumination of poisoned cells is similar to the noise accompanying the very rapid depolarization of receptors evoked by the application of anaesthetics to the surface of the retina (Payne 1980).

4. Cell Input Resistance Drops During Poisoning

The input resistance of photoreceptors in the dark was measured using double-barrelled electrodes (see
5. Sodium-Replacement Experiments

If the voltage noise and the afterpotential recorded from poisoned cells are due to open ionic channels, then it is likely that these channels are permeable to sodium. Replacement of extracellular sodium by choline should therefore abolish, or greatly reduce, the effect that each open channel has on the membrane potential. This is true of the photoreceptors' response to light. Complete replacement of sodium in the perfusion medium by choline reversibly reduces a photoreceptor's response to weak flashes of light by 80%, but fails to abolish it completely (Payne 1980). It was also found that replacement of sodium by choline had no effect on the resting potential.

The normal saline containing 200 mmol sodium chloride (see Methods) was therefore replaced by saline containing 200 mmol choline chloride (and no sodium chloride) immediately after the initiation of the afterpotential that followed a train of flashes delivered to a photoreceptor treated with azide. In all cases the amplitude of the recorded voltage noise was greatly reduced within 60 s of introducing the choline saline (which also contained 1 mmol azide). On reintroduction of saline containing 200 mmol sodium chloride, the voltage noise increased with the same, rapid time course, although it did not always attain its previous amplitude. In 3 out of the 6 cells investigated the depolarization was reversibly reduced, while the depolarization of the other three cells was steady during the removal of sodium, but increased sharply following its reintroduction (Fig. 6). The inability of sodium-replacement to reduce the depolarization in all the experiments probably reflects the tendency of poisoned cells to continue to depolarize after the initial step in potential that follows stimulation by light flashes. This would compete with the ability of the sodium-free saline to reverse the depolarization, but a sudden increase in the depolarization is then to be expected upon the reintroduction of sodium. Qualitatively, then, these experiments support the proposi-
Fig. 5. Upper: Mean voltage change produced by current injected into 4 cells in darkness before azide treatment (open squares); when depolarized 10 mV by 1 mmol azide and 100 ms light flashes at intervals of 20 s (triangles) and after recovery from azide treatment (filled squares). The resistance over the linear portion of the curve measured during azide treatment, $R_n$, was reduced by 32% ± 10% when compared with the resistance, $R$, measured before treatment with azide. Lower: Recordings of a cell's response to a −1 nA current pulse and a flash of light before, during and after treatment with azide in the course of the above experiments. Bottom traces time of flash of light.

Discussion

A dependence of the resting potential on oxidative metabolism is a common feature of small nerve cells. A rapid depolarization of stimulated, anoxic or poisoned photoreceptors has been reported by Baumann and Mauro (1973) and Wong et al. (1976). This depolarization might be thought to arise out of an inability of the poisoned tissue to maintain potassium concentration gradients across the photoreceptors' membranes. However, the observations made in this paper suggest that a leakiness of the receptor's membrane is also important in producing the depolarization seen on poisoning the retina. Analysis of the voltage noise accompanying this leak indicates that it results from the random opening of ionic pathways in the cell's membrane. If the cell is stimulated by light flashes, the depolarization and noise are developed from an afterpotential following each light flash. How does this leakiness arise?

One possibility is that the leak results from the formation of channel-like holes in the photoreceptor's membrane, when the cell runs out of energy. Stimulation of the cell further depletes the energy resources of the cell and so increases the likelihood of these holes, whose formation has no direct linkage with the transduction mechanism. However, since the transduction mechanism has control of the opening of depolarizing ionic channels in the cell membrane, it is also possible that the leak and noise arise from...

Fig. 6. Replacement of perfusate sodium chloride (200 mM) with choline chloride reversible reduces the amplitude of voltage noise recorded following illumination by light flashes of a cell treated with 1 mmol azide. Each record shows a high-gain, a.c. coupled trace and a low-gain, d.c. coupled trace. D.c. coupled traces show that the depolarization is steady during the removal of sodium, but it increases rapidly following replacement of sodium. After wash-out of azide from the perfusate, voltage noise and resting potential recovered completely.
spontaneous activity of the channels normally opened by light. Is there any evidence for this?

The voltage noise recorded in this paper is identical in its amplitude, its spectral shape and in its dependence on external sodium to the noise that accompanies the rapid depolarization of photoreceptors induced by alkanols applied to the retinal surface (Payne 1980). The alkanol-induced noise was shown to be generated by the random opening and closing of channels close to the recording site, at the distal end of the cell. To explain the relationship between alkanol-induced noise and the photon shot noise that was recorded when the cell was depolarized to the same extent by light it was proposed that alkanols activated individual channels that were normally opened by light. At high light intensities (large depolarizations) each photon opens only one channel (Wong 1978) so that alkanol-induced noise and photon shot noise are similar. At low intensities, however, each photon opens several channels to form events (bumps) that are of greater amplitude and are slower than the noise induced by alkanols. These findings are confirmed in Results (3) for the noise recorded from poisoned cells, which ‘mimics’ noise recorded during illumination of high intensity but not of low intensity. At no stage during recovery from azide could individual events, resembling quantum ‘bumps’, be resolved in the noise. This is in contrast to the depolarizing after-potential that follows very intense illumination of photoreceptors in the intact locust eye (Tsukahara and Horridge 1977).

It is possible, therefore, that oxidative metabolism is required to reduce the number of channels that open spontaneously and to inactivate channels that were opened during the response to a flash of light. Transduction may involve the release, from a store, into the cytoplasm of an internal transmitter which opens channels (Cone 1973). If this release is driven by a concentration gradient, due to the low activity of transmitter in the cytoplasm compared with that in the store, then energy is likely to be required to maintain the low concentration of transmitter in the cytoplasm in the face of leakage from the store. Such a proposal has analogies with the mechanism of the release of synaptic vesicles at the neuromuscular junction, where the amplification of the internal transmitter, calcium, is driven by a large calcium concentration gradient across the plasma membrane and is gated by calcium channels. This arrangement allows for great amplification, since one calcium channel may let many calcium ions enter the cytoplasm. However, the requirement for a large concentration gradient means that mitochondrial energy is required to maintain the low internal concentration of calcium. Poisoning of the synapse therefore increases the background noise (spontaneous m.e.p.p. frequency) (Alnaes and Rahaminoff 1975). A more direct analogy may be found in the recent findings of Liebman and Pugh (1980). ATP appears to be essential to the rapid inactivation of the light-activated cyclic GMP phosphodiesterase that is thought to modulate the level of internal transmitter (cGMP) in vertebrate rods. Anoxia or poisoning is likely to rapidly reduce the ATP concentration in such a small cell as the locust photoreceptor and so slow inactivation of bleached rhodopsin through a similar phosphorylation step.

High-sensitivity photomultiplier tubes also suffer from spontaneous noise. The high voltages at the cathode, required to produce sufficient amplification of the signal, introduce thermal noise. Energy must be expended in cooling the photomultiplier to reduce this background activity. It therefore comes as no surprise, perhaps, that the locust photoreceptor, a detector with very high sensitivity, requires active suppression of spontaneous noise.

Whatever the mechanism is which generates the leak in the photoreceptor’s membrane, it is clear that the maintenance of the integrity of that membrane requires the expenditure of energy, especially following exposure to light. This finding complements speculation by Blest (1980) and Harris and Stark (1977) that the photoreceptive membrane, or some transduction apparatus associated with it, ‘ages’ or becomes damaged with use. This work, and the previous work with alkanols suggest that the membrane itself, or the transduction mechanism that opens channels in the membrane, is made unstable following the application of a variety of chemical agents and also following illumination when oxidative metabolism is poisoned by azide or in anoxic conditions.

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CHAPTER THREE:

FLUORIDE BLOCKS AN INACTIVATION STEP OF TRANSDUCTION
IN AN INSECT PHOTORECEPTOR
SUMMARY

1) Photoreceptors in a superfused retina of a locust compound eye are treated with saline containing 10mM NaF, while their intracellular resting potential and responses are recorded using glass micropipettes.

2) Treatment for two minutes with 10mM NaF followed by a series of brief, bright flashes of light, results in an irreversible, noisy depolarization of approximately 10mV. The final, stable level of depolarization is reached through the summed effect of each of the noisy depolarizing afterpotentials that follow every response of the cell to a light flash. If kept in darkness after treatment with NaF, the noisy depolarization still develops, but more gradually, over a period of five minutes.

4) The voltage noise induced by NaF mimics light-induced voltage noise when the two are compared at mean depolarizations of more than 15mV. At very small depolarizations, however, fluoride-induced noise cannot be resolved into the large discrete events (bumps) that are typical of the response of a dark-adapted photoreceptor to a single photon.

5) The complete replacement of the superfusate sodium by choline reversibly reduces the fluoride-induced noise and depolarization to the same extent as it does the light-induced noise and depolarization of an illuminated cell.

6) Increasing the superfusate calcium concentration from 0.5mM to 10mM also reversibly reduces fluoride-induced noise and depolarization to the same extent as it does light-induced noise and depolarization. This action of calcium is accompanied by an increase in a cell's input resistance which opposes the reduction caused by light or fluoride treatment.
7) The results confirm the proposal (Payne, 1981) that anionic metabolic inhibitors cause spontaneous activity in sodium channels that are normally opened by light. A model is proposed in which fluoride acts by blocking the inactivation of a late stage in the transduction mechanism.
METHODS

Details of the preparation, computational and recording techniques are available in chapters 2 and 3.

The preparation

Compound eyes of adult Locusta migratoria were excised and mounted in a perspex chamber of volume 0.7ml. The eye was cut so as to expose photoreceptors at the base of the eye along their entire length distal to the basement membrane of the retina. The cut eye was superfused with oxygenated salines of differing composition which entered the bath through a tap, enabling rapid switching between salines. The half-time of solution changes was approximately 20s.

Solutions

The normal saline used in these experiments was of composition (mM): Na, 200; Cl, 204; K, 3; Ca, 0.5; glucose, 90; TRIS-HCL, 10: pH 7.0. Salines containing increased concentrations of calcium or magnesium were made by replacing sodium chloride with calcium or magnesium chloride so as to retain the solution's osmolarity. Sodium-free salines were produced by substituting choline chloride for sodium chloride. Fluoride was introduced into the superfusion fluid by the addition of 10mM sodium fluoride to the normal saline. Saline containing fluoride was always freshly prepared.
INTRODUCTION

Most invertebrate photoreceptors respond to illumination by depolarizing. This depolarization is accompanied by voltage noise, (light-induced noise) which is caused by the random summation of single photon events (Dodge et al 1968) and the transducer noise described by Lillywhite & Laughlin (1979). Each effective absorption of a photon is thought to cause the opening of one or more sodium channels, depending on the state of light adaptation of the cell (Wong, 1978).

Chapter two of this thesis has described the effects of poisoning photoreceptors in a superfused locust compound eye, using anoxia or azide. Poisoning caused a slow increase in the voltage noise recorded intracellularly from photoreceptors, accompanied by a depolarization. This effect was greatly accelerated by illumination during poisoning, when the response to each flash of light was followed by a sustained, noisy, depolarizing afterpotential. A study of the power spectrum of this noise and the analysis of the noise variance as a function of depolarization, lead to the conclusion that the afterpotential arose from the random summation of depolarizing events of amplitude 40µV. The azide-induced noise also had a similar amplitude and time course to light-induced noise recorded during strong steady illumination of a light adapted photoreceptor. It was proposed that the azide-induced noise resulted from the residual, random activation of ionic channels that had been open during the response to flashes of light. Metabolic energy might therefore be required to turn off the transduction process, once initiated.
Such speculation about the role of metabolic energy in transduction could be supported by a more positive identification of the source of azide-induced noise. This identification requires stable levels of poison-induced noise and the accompanying depolarization. The effects of channel blocking agents and ion replacement on the poison-induced noise could then be compared with their effects on light-induced noise. A stable condition was not attainable using azide because its effects were too quickly reversed. Continued application of azide resulted in a slow, steady, increase in depolarization following the light flashes, while removal of azide caused a return of the noise and cell potential to normal levels within 5 to 10 minutes. Fein and Corson (1979) reported that *Limulus* ventral photoreceptors exhibit increased spontaneous noise when treated with 10mM NaF. Following their work, I report that fluoride acts similarly to azide on *Locusta* photoreceptors, causing a noisy depolarization. The effect of fluoride is, however, irreversible. Fluoride can therefore be applied before illumination and washed out of the superfusing saline. The afterpotential can then be induced by light flashes and it will be maintained for at least as long as the cell can be stably impaled on a micro-electrode (\( \frac{3}{4} \) hr).

This paper applies a variety of tests to show that the fluoride-induced noise and light-induced noise arise from the transduction mechanism. Both types of noise are reduced to the same extent during the complete replacement of superfusate sodium by choline. In addition, external calcium ions modulate the amplitude of the fluoride-induced noise in the same way as they modulate the light-induced noise. The modulation by calcium of
the response to light is thought to mediate light adaptation in invertebrate photoreceptors (Millecchia & Mauro, 1969; Lisman & Brown, 1972). However, in contrast to the results obtained from Limulus by Fein & Corson (1979), it is clear that the fluoride-induced noise recorded from Locusta photoreceptors cannot be resolved into events as large as those that constitute the response of a dark adapted cell to a quantum. Instead, the noise may arise from spontaneous activity at a late stage in transduction. The results of this paper provide further evidence for the role of metabolism in turning off the response of a photoreceptor to light and in reducing the spontaneous activity of the transduction mechanism. The findings complement the recent work of Bolsover & Brown (1981) and Fein & Corson (1981) on Limulus ventral photoreceptors in which a requirement for GTP to reduce spontaneous noise has been suggested by experiments using GTP analogues.
RESULTS

The initiation of the fluoride-induced depolarisation.

Locust photoreceptors were penetrated with glass micropipettes. When their resting potential was stable, the normal saline perfusing the preparation was replaced by saline to which 10mM NaF had been added. Perfusion with fluoride was continued in darkness for approximately 120s, after which time perfusion with the normal saline was resumed. Over this period of treatment with NaF, the r.m.s. voltage noise recorded from the photoreceptors rose from $100\mu V \pm 34\mu V$ to $182\mu V \pm 53\mu V$ ($n = 11$; a significant increase at the 0.5% level). No significant depolarization could be detected. Although the voltage noise increased, individual events could not be resolved.

If kept in darkness following the resumption of perfusion with normal saline, the cells depolarized to a steady level of 10 to 20mV over a period of about five minutes. This depolarization was accompanied by a large increase in voltage noise. Alternatively, to induce the noisy depolarization immediately, a series of brief, bright flashes of light were delivered to the preparation. Each response to a flash of light was followed by a small, sustained, noisy depolarizing afterpotential (fig. 1). The flashes were continued until the depolarization arising from the sum of the individual afterpotentials following each flash was approximately 10mV. This required between 3 and 10 flashes. The cell was then left in darkness for five minutes, during which time a further small depolarization developed in some cells and the stability of the penetration could be judged. Following the period of stabilization, the r.m.s. voltage noise was $523\mu V \pm 175$
Figure 3.1
Receptor potentials recorded from a locust receptor in normal saline (left hand response) and following treatment for 140s with 10mM NaF and a return to normal saline for 20s. Note the noisy afterpotential following the first flash of light after NaF treatment. The flash of light (lower trace) was of intensity $9 \times 10^{13}$ photons.cm$^{-2}$.s$^{-1}$, wavelength 560nm and duration 10ms. The two flashes delivered after NaF treatment were 5s apart. The potential shown is relative to the cell's potential in the dark at the beginning of the experiment.
μV, superimposed upon a depolarization of 9mV ± 5.2mV. The depolarization and accompanying noise continued for as long as the cell could be held on the electrode, up to 3/4 hour after the initiation of the afterpotential. Once the depolarization was stable, further stimulation of the cell did not result in further depolarization or increase in noise. No matter how the final depolarization was reached, following light flashes or by leaving a cell in darkness for a period, there was no qualitative difference in the amplitude and time course of the noise produced. This suggested that the effect of illumination was to greatly accelerate the approach to a stable, noisy depolarization through a series of afterpotential steps and that the underlying mechanism is largely but not completely dependent on light for its initiation.

These observations are similar to those made in chapter two of this thesis of the noise recorded during treatment of locust photoreceptors with the reversible metabolic poison azide. A quantitative analysis of the voltage noise

To quantify the relationship between fluoride-induced noise and light-induced noise, difference power spectra were calculated from digitized, a.c. coupled potentials recorded before and following fluoride treatment. The power spectra of light-induced noise were calculated from sections of the response of a photoreceptor to 20s pulses of steady illumination. The receptors were initially dark adapted. The intensity of the illumination during each 20s pulse was increased so that power spectra could be obtained at light-induced mean depolarizations of 0.5mV to 20mV. The photoreceptor was allowed to dark-adapt and
Figure 3.2

A comparison of the amplitude and power spectra of fluoride-induced noise with light-induced noise recorded at the same depolarization. At a depolarization of 15mV, the two noise types match in amplitude and power spectrum (top, right). At a depolarization of 2.5mV, the fluoride-induced noise is faster and of less variance than light-induced noise. The power spectrum of fluoride-induced noise falls less rapidly at high frequencies than that of light-induced noise when compared at a depolarization of 2.5mV (bottom right). Power spectra were calculated from ten 2s segments of the noise following fluoride treatment or during illumination. A spectrum obtained from the cell before fluoride treatment and after illumination (bottom trace) was subtracted from the noise spectra and the result normalized for comparison. The light intensities used were $3 \times 10^{13}$ photons.cm$^{-2}$.s$^{-1}$ (15mV) and $7 \times 10^{11}$ photons.cm$^{-2}$.s$^{-1}$ (2.5mV) 20°C.

Figure 3.3

Fluoride-induced voltage noise variance plotted as a function of the mean fluoride-induced depolarization during the first five minutes after a photoreceptor had been treated for 130s with 10 mM NaF and then had been left to depolarize slowly in darkness. Triangles show the raw data, circles are data corrected for non-linear summation (see text). The line through the latter data is the line of linear regression.
2.

\[ \text{LIGHT} \]
\[ 15 \text{mV} \]
\[ F^- \]
\[ 250 \text{ ms} \]

\[ \text{LIGHT} \]
\[ 2.5 \text{mV} \]
\[ F^- \]
\[ 2 \text{mV} \]

\[ \text{BEFORE} \]
\[ F^- \]
\[ 250 \text{ ms} \]

3.

<table>
<thead>
<tr>
<th>DEPOLARIZATION</th>
<th>VARIANCE</th>
</tr>
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<tr>
<td>0</td>
<td>0.15</td>
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<tr>
<td>3</td>
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<td>0.60</td>
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\[ \begin{align*}
\text{LOG } S(f) & \quad \text{LOG } S(f) \\
\text{F}^- & \quad \text{F}^- \\
\text{LIGHT} & \quad \text{LIGHT}
\end{align*} \]
it was then treated for approximately 120s with 10mM NaF. Following the treatment with NaF, the cell remained in darkness and power spectra were again calculated as the cell slowly depolarized and became noisy over the next five minutes. Power spectra obtained during illumination were compared with the spectra obtained from the cell when depolarized to the same extent following fluoride treatment.

At small depolarizations, fluoride-induced noise is both faster and of smaller amplitude than light-induced noise (fig.2). As noted in section 1, no individual depolarizing events were resolvable in the fluoride-induced noise, whereas light-induced noise can easily be resolved into discrete bumps of amplitude 1-5mV at low light intensities. As the depolarization at which fluoride-induced noise is compared with light-induced noise is increased, the amplitude of light-induced noise declines relative to that of fluoride-induced noise so as to equal it, for the cell of fig. 2, at depolarization of more than 15mV. Their power spectra are also identical (fig.2). This similarity of noise power spectral shape and amplitude was also true of six other cells depolarized to 12mV ± 2mV by strong, steady, light (\( >10^{12} \text{photons.cm}^{-2}.\text{s}^{-1} \)) and subsequently to 10.5mV ± 4mV by fluoride treatment. The power spectra of the noise declined in amplitude as approximately \( 1/f^3 \) at high frequencies.

The equivalence of fluoride-induced and light-induced noise at depolarizations of 10mV contrasts with those cases in which a comparison was made at depolarizations of <5mV, i.e. during dim illumination and during the early stages of fluoride induced depolarization in darkness. In addition to the very much greater
amplitude of the light-induced noise, the power spectrum of the noise rolled off at a rate that was approximately $1/f^2$ faster than that of the spectrum of fluoride-induced noise (fig. 2). The exact shape of these power spectra will depend on the current noise and also on the electrical filter properties of the retinula cell, a cable of complex structure which has a time constant of approximately 10ms (chapter 1).

Quantitative consideration of these parameters in order to explain the $1/f^3$ spectral roll-off of F$^-$ induced noise and the changes in spectral shape with depolarization are beyond the scope of the present paper. However, if we make the simplifying assumption that fluoride treatment does not affect the passive properties of the electrical filter represented by the membrane, then our conclusion from the power spectral data of this section is that the current events underlying light-induced noise become smaller and faster (Dodge, Knight & Toyoda, 1968) as light intensity is increased so as to eventually become indistinguishable from the fluoride-induced events.

The amplitude of the fluoride-induced event

In chapter 2, on page 43, the amplitude of the azide-induced event was estimated by applying the analysis of Katz & Miledi (1972). A similar analysis can be applied to the fluoride-induced noise by measuring the increase in noise variance during the slow depolarization that follows fluoride-treatment if the cell is kept in darkness (fig. 3). In the example illustrated, a small depolarization of approximately 1mV developed during the period of treatment with fluoride (120s), when little increase in noise variance occurred. However, following the
switch back to normal saline, the noise variance rose smoothly as the cell continued to depolarize. The variance, when corrected for the non-linear summation of voltage events, is linearly related to the depolarization of the cell. The slope of this linear relation gives an estimate of the event size, which is 60µV in this case, assuming an exponential event shape. Thus the fluoride-induced event is of similar amplitude to that induced by azide (approximately 40µV). The small depolarization recorded from the cell during fluoride-treatment may be attributable to drift in the cell's potential. As reported above, for a large sample of cells, no significant mean depolarization could be detected during fluoride treatment.

Sodium replacement reduces light-induced noise and fluoride-induced noise.

Complete replacement of the perfusate sodium by choline reduces the response of an insect photoreceptor to flashes of light but does not abolish it (Fulpius & Baumann, 1969; chapter one). Sodium replacement has no effect on the membrane potential of locust photoreceptors in darkness, but reduces the depolarization and noise recorded on steady illumination (chapter one).

In order to compare the effects of sodium replacement on light-and fluoride-induced noise, cells were first depolarized by about 10mV using steady illumination. Sodium-free saline was then introduced. The decline in the noise amplitude and the depolarization were photographed from high gain a.c. coupled and low gain d.c. coupled oscilloscope traces (Fig. 4). Following the re-introduction of saline containing sodium, and the recovery
Figure 3.4

The upper two traces show a high gain, a.c. coupled recording and a low gain d.c. coupled recording of the reduction, during sodium replacement by choline, of the voltage noise (high gain) and depolarization accompanying steady illumination of a locust photoreceptor. Illumination began at the star marked on the record. Below the d.c. trace are samples recorded using a faster time scale. The recordings were made (left to right) before illumination; then during illumination in sodium saline; choline saline; and back into sodium saline.

The lower set of records show the same cell treated with 20mM NaF so as to initiate a noisy depolarization. The section of record to the left of the star shows the noise and membrane potential recorded before NaF treatment, while the record to the right of the star shows the fluoride-induced noise and depolarization and the effect of sodium replacement. As with illumination, sections of record on an enlarged time base are shown (left to right) before fluoride treatment: after initiation of the fluoride-induced noise in sodium saline; choline saline; and once more in sodium saline.

The intensity of light used for the top record was $3 \times 10^{12}$ photons $\text{cm}^{-2}\text{s}^{-1}$, wavelength 560nm.
of the noise levels and the depolarization, illumination was stopped and the cell was treated with fluoride so as to induce the noisy afterpotential described on page 56. When this depolarization was stable, sodium replacement was repeated as before.

Measurements of the amplitude of the voltage noise and the mean depolarization were calculated from digitized traces (see Methods). Sodium replacement reversibly reduced the light-induced depolarization of 4 cells from 12mV ± 2mV to 5mV ± 1mV while the increase in the amplitude of the voltage noise due to illumination fell from 640μV ± 210μV to 240μV ± 40μV. The fluoride-induced depolarization of the same cells was reduced from 9mV ± 4 mV to 2.6mV ± 2.4 mV while the voltage noise fell from 590μV ± 200μV to 190μV ± 40μV. The magnitude of the effects of sodium replacement on light-induced and fluoride-induced noise are therefore similar. In addition, for any one cell, the time courses of the decrease in the noise and the depolarization are also similar (fig.4).

High calcium reduces light-induced noise and fluoride-induced noise

The concentration of calcium inside invertebrate photoreceptors is thought to regulate the changes in the time scale and the sensitivity of the receptor's response that characterize the adaptation of the cell's response to bright light (Millecchia & Mauro, 1969; Lisman & Brown, 1972; Bader et al 1976; Fein & Charlton, 1977). Rather than raising the internal calcium concentration directly by injecting calcium through micropipettes, Martinez & Srebro (1976) have shown that increasing the
Figure 3.5

Upper left: Receptor potentials recorded in response to weak, brief flashes of light, delivered at 1.5s intervals before (DA) and 9s after (LA) a bright, adapting flash of light. The test flash of intensity $3 \times 10^{13}$ photons cm$^{-2}$ s$^{-1}$, duration 1ms, was delivered at the beginning of the traces.

Upper right: Responses from the same cell to the same intensity and duration of flash in normal saline (0.5 mM Ca) and 40s after a switch to superfusion with high-calcium saline (10 mM Ca).

Below: The cell was illuminated with steady light and then treated with high calcium saline. (a) was recorded before illumination; (b) during illumination in normal saline; (c) 60s after switching to high calcium saline (10 mM Ca) while continuing the illumination; and (d) 4 minutes after reverting to normal saline. The light intensity used was $3 \times 10^{12}$ photons cm$^{-2}$ s$^{-1}$. 
concentration of calcium in the saline bathing the receptor has similar effects to injection. Maaz & Stieve (1980) suggest that calcium enters Limulus ventral photoreceptors either during their response to light or that exchange of calcium occurs between the cytoplasm and the external saline. Experiments were therefore performed on locust photoreceptors to verify that the elevation of the superfusate calcium concentration can adapt a cell's response to light and then to see whether a similar adaptation occurs in the response to fluoride occurs.

A twenty-fold increase of the calcium concentration in the superfusate, from 0.5 mM to 10 mM, reduced the time to peak of the response of six photoreceptors illuminated by brief, weak flashes from 60ms ± 7 ms to 42ms ± 3 ms (Fig. 5). The area under these responses diminished to 41% ± 18% (range 25% to 75%) of its original value. A small depolarization of 4mV ± 3 mV (range 0 to 9 mV) also developed in high calcium saline. These changes were complete within 90s of switching the salines, so that, if the site of action of calcium is inside the cell, the calcium must penetrate the cell rapidly. The effects of high calcium are reversed within 10 minutes of returning to perfusion with normal saline.

To compare the effects of high external calcium with light adaptation the same photoreceptors were adapted by a bright flash of light lasting 2s. Following this adapting flash, the responses to a series of dim flashes, of the same intensity as was used during the experimentation calcium were recorded at 1.5s intervals. As dark adaptation was regained, the amplitudes of these test responses and their times-to-peak increased. From
this set of responses one was chosen which had the same amplitude as those recorded during perfusion with the high calcium saline. The time to peak of the selected light-adapted responses was 34ms ± 5ms and was in every case less than that recorded in high calcium salines (α<0.005; paired t-test). Thus high external calcium can qualitatively mimic the effect of an adapting light flash but, for the same decrease in response amplitude, it cannot quantitatively match the decrease in latency.

As might be expected from its effect on the response to light flashes, high external calcium concentrations reversibly reduced the depolarization and the extra voltage noise associated with steady illumination of the photoreceptors. The r.m.s. noise amplitude was reduced from 700µV ± 200µV to 250µV ± 100µV within 90s of switching to high calcium saline, while the mean light-induced depolarization dropped from 11mV ± 3 mV to 2.4mV ± 4.6mV (n = 6) (Fig.6). Some cells hyperpolarized to beyond their initial resting potentials in the presence of light and high calcium saline. Hyperpolarizations were often observed upon the cessation of strong illumination during perfusion with normal saline (for example, the light-adapted response of fig. 5). The hyperpolarization of some illuminated locust photoreceptors in high-calcium saline may therefore be due to potassium conductances opened by light or to the activity of electrogenic pumps rather than to any action of calcium. In darkness, high calcium saline had a slight depolarizing action on the cell (fig.5) which is to be expected if, for instance, the cell is slightly permeable to calcium in darkness.
Figure 3.6

The upper two traces show a high gain a.c. coupled and a low gain d.c. coupled recording of the reduction, during perfusion with high (10 mM) calcium saline, of the voltage noise and depolarization accompanying steady illumination of a locust photoreceptor. Illumination began at the star marked on the record. Below the d.c. trace are samples recorded using a faster time scale. The recordings are made (left to right) before illumination; then during illumination in normal (0.5 mM Ca) saline; high calcium saline; and following a return to normal saline. The lower set of records show the same cell treated with 10 mM NaF so as to initiate a noisy depolarization. The section of record to the left of the star shows the noise and membrane potential recorded before NaF treatment, while the record to the right of the star shows the fluoride-induced noise and depolarization and the effect of high calcium saline. As with illumination, sections of record on an enlarged time base are shown (left to right) before fluoride treatment; after initiation of the fluoride-induced noise in normal saline; high Ca saline; and once more in normal saline.

The intensity of light used for the top record was 3 x 10^{12} photons cm^{-2} s^{-1}, wavelength 560 nm.
After the cells had recovered from high-calcium saline, the light was switched off and they were treated with fluoride and then with light flashes, as described on page 56, so as to induce the noisy afterpotential. When the depolarization associated with this afterpotential had stabilized, the cells were once again bathed in saline containing 10 mM rather than 0.5 mM calcium. The depolarization of these cells, relative to their potential before treatment with fluoride, dropped from 10.5 mV ± 5 mV to 4.4 mV ± 4 mV, while the voltage noise amplitude dropped from 470 µV ± 130 µV to 200 µV ± 70 µV. For a given cell this reversal of the depolarization and the noise amplitude occurred with the same time course as had the effect of high-calcium on the response to steady illumination (Fig. 6). A paired t-test carried out on the relative depression of voltage noise recorded during the action of calcium on light- and fluoride-induced depolarization of the same cell showed no significant difference for the six cells.

Although high calcium salines reduced the amplitude of light-induced or fluoride-induced noise, no significant change was observed in the shape of the power spectra of the noise recorded in high calcium saline as compared with those recorded in normal saline. This observation contrasts with the reduction in the time scale of the dark-adapted response when high-calcium salines were applied.

Replacing the initially magnesium-free saline with one containing 10 mM magnesium had little effect on the light-induced or fluoride-induced depolarization. The r.m.s. voltage noise amplitudes were reduced to 86% ± 13% and 90% ± 13% of their respective original levels while no significant reversal of the
depolarization could be detected. Magnesium cannot, therefore, substitute for calcium in this process, a conclusion also reached by Martinez & Srebro (1976) in their study of response latency changes in Limulus ventral photoreceptors. Calcium antagonizes the opening of channels opened by light or fluoride.

Photoreceptors were penetrated with 0 glass electrodes of resistance 100-200MΩ. Electrical potential was recorded from one barrel, while current was passed down the other. The electrical coupling between barrels was less than 10% of a given cell's input resistance (see Methods).

Two experiments were performed. In the first, a fluoride-induced depolarization was initiated in an impaled photoreceptor. Once the depolarization was stable, current was injected for 10s, so as to alter the cell's membrane potential, while the voltage noise amplitude and the mean depolarization were calculated from digitized traces (Fig.7). Hyperpolarization increased the voltage noise, while depolarization reduced it. This behaviour is qualitatively consistent with the noise having arisen from the opening of ionic channels with a reversal potential that is 40-50mV more positive than the resting potential of the cell in darkness. The currents used, <2nA, did not produce significant artifactual noise in the recording electrode when injected into the cell in darkness prior to fluoride-treatment. However, the artifactual noise amplitude rose rapidly when injecting more than 3nA so that the 'reversal potential' of the fluoride-induced noise could not be directly demonstrated.
Figure 3.7

The relationship between the relative variance of the increase in voltage noise \( \frac{\sigma v}{\sigma v_0} \) created after exposure to 10mM NaF, and the membrane potential as recorded during 10s injections of current into the cell through 9 glass electrodes.

To the left of the graph are shown the records from which the graph was calculated. The samples shown were taken before fluoride treatment; during the fluoride-induced depolarization with no current injected (PDA); and when injecting ± 2 nA during the fluoride-induced depolarization. The samples were of 3 s duration, digitized by a PDP8-E computer at a frequency of 333 samples per second and subsequently plotted out by a digital graph plotter.
The second experiment was designed to demonstrate the mode of action of calcium in reducing the response to light. Given what is known about the action of calcium on membrane conductance, it was possible that calcium was acting either by opening potassium channels (Hanani & Shaw, 1977) and hence shunting the light-induced changes in membrane resistance, or by directly antagonizing the opening of depolarizing channels by light. In the former case the action of calcium on an illuminated cell would be accompanied by a decreased cell input resistance, while in the latter the input resistance would increase so as to return towards its value in darkness.

Fig. 8 shows the results of an experiment in which voltage-current relationships (V-I curves) were recorded from a cell in darkness, when illuminated in saline containing 0.5mM calcium and following the increase of the calcium to 10mM while illumination continued. The voltage noise and the depolarization induced by light are abolished by the high calcium saline, and the membrane resistance increases so as to equal that recorded before illumination. Thus calcium acts so as to block the light-induced conductance increase, which accords with voltage clamp data from *Limulus* ventral photoreceptors (Lisman & Brown, 1972; Fein & Charlton, 1977).

Figure 8 also shows the result of the measurement of V-I curves during the action of calcium on the noisy depolarization induced by fluoride. The cell resistance again increases to its value before fluoride treatment as the high calcium saline reduces the noise and depolarization of the cell. The action of calcium on the light and fluoride-induced depolarizations cannot be
Figure 3.8

Relationships between the voltage recorded during current injection into photoreceptors through 6 glass electrodes and the current amplitude (V-I curves).

Cell 1: V-I curves are shown before illumination (circles); during illumination of intensity $3 \times 10^{12}$ photons.cm$^{-2}$.s$^{-1}$ in normal saline (squares); and 60s after the introduction of saline containing 10 mM Ca, while illumination continued (triangles). The traces a-d show the data from which the V-I curves of cell 1 were plotted. The response to a -0.25 nA pulse of current is shown (a) before illumination; (b) during illumination in normal saline; (c) during illumination in high-calcium saline; and (d) following the re-introduction of normal saline.

Cell 2: V-I curves are shown before treatment with 10 mM NaF (circles), following the initiation of a noisy depolarization by NaF and light flashes (squares) and 60 s after introducing high calcium saline during this depolarization (triangles). (e)-(h) show the voltage recorded during current pulses of amplitude - 0.5 nA; (e) before NaF; (f) during the fluoride-induced depolarization in normal saline; (g) during the fluoride-induced depolarization in high calcium saline; and (h) following return to normal saline.
attributed to a shunting of the membrane resistance by calcium-activated potassium channels. Calcium appears to block the mechanism by which both treatments open ionic channels in the membrane.

**Bright light adapts the fluoride-induced noise**

If calcium, the agent thought to mediate light-adaptation, is able to block the noise induced by fluoride, it might be expected that a temporary reduction in noise amplitude and depolarization will follow bright flashes of light delivered when the fluoride-induced depolarization is stable. The interpretation of such an experiment is open to doubt. Treatment with NaF may effect the release of calcium into the cells cytoplasm by light. Flashes delivered after fluoride treatments may also show noisy depolarizing 'tails' which will mask their 'adapting' effect. A preliminary experiment (fig. 9) shows that, qualitatively, the fluoride-induced noise and depolarization are reduced following a bright flash of light and that the membrane resistance increases slightly during this 'adaptation'. Further work will quantify these changes and compare them to light adaptation.

**Effects of ouabain and EDTA on voltage noise**

If fluoride acts on photoreceptor membranes as it does on those of the squid axon and the red blood cell (Canessa-Fischer et al, 1968; Whittam, 1964), then it may block the active transport of sodium and potassium. The consequent decline in the concentration gradients of sodium and potassium would both depolarize and reduce the response of a photoreceptor (Smith et al, 1968; Stieve,
Figure 3.a

a) High gain a.c. and low gain d.c. coupled recordings from a 0 glass electrode impaling a photoreceptor before treatment with NaF (far left) and during the subsequent fluoride-induced depolarization. At the arrow a saturating white light flash was delivered to the photoreceptors from a microscope lamp. Following the response to the flash, the fluoride-induced noise and depolarization are reduced.

b) The flash of light was repeated while injecting square pulses of current (0.5 nA) down the other barrel of the 0 glass electrode. The cell's resistance is almost abolished during the flash, but following the light flash, the resistance of the cell is slightly increased.
1973). Following the introduction of 1mM ouabain to the normal saline perfusing the locust retina, photoreceptors depolarized in darkness by 6mV ± 1.2mV (n = 6) within two minutes. Brief, bright flashes of light delivered subsequently, failed to induce the sustained increase in the voltage noise recorded from the cells in darkness that similar flashes would have induced in cells that had been treated for the same length of time with fluoride. The receptors continued to depolarize in ouabain after the flashes and this depolarization was not reversible on removing ouabain from the saline.

Fluoride could also act by precipitating magnesium and calcium in the preparation during the period of its application because both CaF$_2$ and MgF$_2$ are insoluble. This possibility was tested by adding 2mM EDTA to the perfusing saline and so removing calcium and magnesium. Within two minutes of starting perfusion with saline containing EDTA a five fold increase in the time-scale of the response to a bright flash was observed, but no increase in the amplitude of the voltage noise recorded in darkness. Also, note that fluoride, unlike EDTA, causes little change in the time-scale of the cell's response (fig. 1).

Thus reagents which mimic two possible actions of fluoride do not produce the observed noise increase. It is unlikely that fluoride is inducing noise either via an action on the sodium pump or by reducing the concentration of free calcium.
DISCUSSION

Locust photoreceptors exhibit little spontaneous noise in darkness (Lillywhite & Laughlin, 1979). There are less than 10 spontaneous single photon events per hour and the continuous voltage noise is negligible when compared with dark-adapted single photon events ("bumps") of amplitude 1-5mV. Treatment with the metabolic inhibitors azide (chapter two) or fluoride results in a dramatic rise in continuous voltage noise. The present paper tests the possibility that this noise arises from a malfunction of the transduction mechanism.

The azide noise reported previously has a similar amplitude and power spectrum to fluoride-induced noise recorded at comparable depolarizations. The method of inducing the noise, using light flashes, is also similar. The effects of azide are, however, reversible within 10-15 mins. of removing azide from the bathing saline, while fluoride appears to act irreversibly. It will be assumed in this discussion that both fluoride and azide act on the same process to produce the noise characteristic of both treatments and the conclusions of the previous paper on azide will be applied together with those of the present paper in order to explain the production of noise. The following observations must be explained:

1) The random nature of the noise and the size of the underlying shot event.

2) The afterpotentials that follow flashes of light delivered after treatment with fluoride.

3) The slow continuous rise of the noise amplitude during fluoride treatment in darkness.
4) The ability of fluoride induced noise to mimic light-induced noise recorded from photoreceptors depolarized more than 15mV by illumination even though at very small depolarizations the fluoride-induced noise cannot be resolved into individual events resembling the bumps of light-induced noise. The ability to mimic light-induced noise extends to the effect of sodium replacement and high-calcium salines, which reduce the amplitude of fluoride-induced and light-induced noise and depolarization to the same extent.

Figure 10 describes a model that qualitatively describes the action of fluoride and azide. The model follows Wald (1965) and Borsellino et al (1965) in describing transduction as a cascade of enzymes. The cascade model has obvious difficulties when applied to insect photoreceptors due to the large number of stages required to fit the long delay before a response occurs (Payne & Howard, 1981). Therefore, following Wong et al. (1980) and Tiedge (1981), the model of fig.10 contains a non-amplifying, latency-generating process followed by a short, amplifying cascade. Each enzyme in the cascade is active for a brief period so as to produce, at the end of the cascade, a burst of transmitter in response to the photoisomerization of a single rhodopsin molecule.

The transmitter randomly activates ionic channels in a similar manner to transmitters acting at neuromuscular junctions (Katz & Miledi, 1972). Fluoride blocks the inactivation of an enzyme in the cascade so that, once it is activated by light, the enzyme will then continue to stimulate the cascade below it, causing an afterpotential. If the affected enzyme is early in
Figure 3.10

A scheme for phototransduction whereby the effects of fluoride might be explained. The absorption of a photon by rhodopsin causes the conversion of rhodopsin to an active metarhodopsin, M-R* (Hamdorf & Kirschfeld. 1980). M-R* initiates an enzyme cascade after a delay time $\Delta t$. The cascade results in the production of a burst of active "internal transmitter", T*. It is suggested that fluoride blocks the inactivation of a later stage in transduction, for example the conversion of T* to its inactive form $T_1^i$ or $E_2^*$ to $E_2^i$. 
the cascade, the transmitter will be released in bursts, resembling those that produce a bump. A less correlated release of transmitter will result if the affected enzyme is at a late stage in the cascade. A shot noise analysis has been applied to azide-induced noise during the slow recovery of membrane potential when azide was removed from the bathing saline (chapter two). On page 27 a similar analysis has been applied to fluoride-induced noise. The predicted shot event amplitudes, 40\mu V (azide) and 60\mu V (fluoride) correspond to a conductance increase of less than 60pS, which is small enough to be due to the opening of single ionic channels (Neher & Stevens, 1977). There is, however, no direct evidence of single channel kinetics from the power spectral shape of the fluoride-induced noise and the event amplitude is in the upper range of expected conductances for single channels.

Power spectral analysis of fluoride-induced noise (fig. 2) shows it to be faster than light-induced noise at very small depolarizations. These characteristics can be explained if fluoride and azide block the inactivation of an enzyme at a late stage in the cascade of fig.10 or block the inactivation of the transmitter itself. The result of such a blockage will be a residual spontaneous release of individual transmitter molecules following the cessation of illumination, causing events that more closely resemble single channel openings and which are smaller and faster than the dark-adapted bump. The faster high frequency roll-off of the power spectrum of fluoride induced noise, compared with dark-adapted, light-induced noise (summed bumps), is also predicted because fewer amplifying stages are involved in the production of fluoride-induced noise. By ascribing the small,
fast events induced by fluoride to spontaneous activity at a late stage in transduction, we are using a similar argument to that of Baylor et al (1980) when explaining the continuous current noise recorded from toad rod outer segments. We should also note the contrast of the results of this paper with the report by Fein & Corson (1979). Using Limulus ventral photoreceptors they show an increased frequency of large, spontaneous events, resembling single photon events, when the photoreceptors are bathed in a saline containing 10mM NaF. Fluoride may attack an earlier stage of transduction in Limulus than it does in locust photoreceptors.

The mimicry by fluoride-induced noise of light-induced noise recorded at large depolarizations can be explained by the theory of Wong (1978). Wong proposes that a light-adapted photoreceptor responds to a single photon with the opening of only one ionic channel, as opposed to the many that open concertedly to form a bump in the dark-adapted state. Light-induced noise will then mimic the events produced following fluoride treatment if the latter are caused by the spontaneous opening of individual channels. The two types of noise are identical when compared at depolarization of greater than 10-15mV, requiring light intensities of >10^{12} photons.cm^{-2}.s^{-1} or >10^3 effective photons per second. This light intensity corresponds well with that of adapting backgrounds that maximally shorten the time scale of the receptor's impulse-response (Howard in press). Thus the receptor is temporally fully light-adapted in the range where the spectra of fluoride-induced noise and light-induced noise match, as is required by Wong's theory.
If the cell is temporally fully light-adapted at light-induced depolarizations of more than 10mV, (although the sensitivity continues to fall with increasing background light-intensity (Howard, in press) then high calcium salines would not be expected to affect the time-scale of the light-induced noise accompanying such depolarizations, but the amplitude of the noise would be reduced. Neither would high calcium salines be expected to alter the time scale of fluoride-induced noise. This explains why no change in the power spectral shape of the noise was observed during the action of high calcium salines. To reduce the fluoride-induced noise, calcium must act on the cascade of fig. 9 at a later stage than that affected by fluoride, so as to reduce the probability of a transmitter molecule opening a channel. However, other sites of action of calcium must also exist, for instance on the latency generating process so as to reduce the response latency (Martinez & Srebro, 1976).

To explain the slow rise in the noise amplitude in darkness following fluoride treatment, we must assume some spontaneous activity of the enzyme that fluoride affects. The assumption of this activity means that we would expect to see some spontaneous release of transmitter even in healthy cells. This release cannot be great because the voltage noise recorded from healthy cells in darkness is low. However, Laughlin & Lillywhite (in prep) comment that 'baseline noise' recorded from locust photoreceptors is in excess of that expected from the recording microelectrode.

The effects of fluoride or azide can therefore be explained by a modification to a late stage in the transduction process. We must now consider how this modification is achieved. Both
fluoride and azide reduce oxidative metabolism. Azide inhibits
the respiratory chain, while fluoride blocks glycolysis (Hewitt &
Nicholas, 1965). Anoxia also causes similar voltage noise to
that induced by fluoride and azide but with less consistency, from
cell to cell. All three methods of metabolic inhibition could
therefore produce noise through a reduction in the concentrations
of intracellular high energy compounds such as ATP or GTP.
However, the "cleanness" and irreversibility of the effect of
fluoride, at least, makes this explanation seem implausible.
Alternatively, fluoride may act directly to inhibit an energy-
requiring enzyme involved in transduction. Experiments by
Bolsover & Brown (1980) and Fein & Corson (1981) indicate that
fluoride may attack, in Limulus ventral photoreceptors, a GTP-ase
so as to produce spontaneous noise. Although there appear to be
differences in the kinetics of the fluoride induced noise in
Limulus and locust, the possibility that a similar system is
involved, or that a similar requirement exists for another high
energy compound such as ATP (c.f. Liebman & Pugh, 1980), is
nevertheless attractive.
CHAPTER FOUR:

NEUTRAL ANAESTHETICS INDUCE A RAPID, NOISY DEPOLARIZATION
OF LOCUST PHOTORECEPTORS
SUMMARY

1) Chloral hydrate and alkyl alcohols induce a rapid, reversible depolarization of superfused locust retinula cells when dissolved in the perfusing saline.

2) Analysis of the voltage noise that accompanies the depolarization induced by chloral hydrate suggests that depolarizing ionic pathways are being randomly opened. Replacement of perfusate sodium by choline reversibly reduces the effects of chloral hydrate, suggesting that the ionic pathways opened are permeable by sodium.

3) The voltage noise induced by chloral hydrate is in many respects similar to that seen following illumination of a fluoride-treated photoreceptor. It is proposed that chloral hydrate causes the rapid spontaneous activation of the light-sensitive channels, possibly through the release of 'internal transmitter'.

4) Like light, chloral hydrate is able to initiate a noisy afterpotential in a fluoride-treated photoreceptor.
INTRODUCTION

For this chapter, measurements were made of the electrophysiological effects on locust retinula cells of alkyl alcohols and chloral hydrate. These compounds were dissolved in the perfusing saline at concentrations that have been noted as having an anaesthetic effect when applied to other tissues (Armstrong and Binstock, 1964; Seeman, 1972; De Felice and Alkon, 1977). The original intention of the experiments was to try to discover compounds that would block the response to light either by acting on the light-sensitive channels, or by acting on the mechanism that opens them. This intention was encouraged by the ability of these compounds to decrease the excitability of axon and statocyst preparations (Armstrong and Binstock, 1964; De Felice and Alkon, 1977). The results of this chapter were surprising, in that these neutral anaesthetics appeared to be able to activate, rather than inactivate, a stage in transduction. The effects of alcohols reported in this chapter are also intended as control results to help recognize, in future experiments, anaesthetic side-effects of drugs that are usually specific to a particular biochemical pathway. These anaesthetic effects may be important when applying high concentrations of lipid-soluble drugs in the medium bathing the retina in an attempt to modify transduction.
Chemicals

Octan-1-ol, butan-1-ol and chloral hydrate were of analytic quality. When dissolved in saline, none of these chemicals had any effect on electrode tip potentials, indifferent electrode potential or on voltage noise. Drugs were generally applied by solution in the perfusing saline. In some experiments, drugs were applied directly onto the surface of the retina via a micro-syringe A broken glass micropipette, with a tip diameter of 50-100 μm, was connected to a motor-driven syringe filled with 100 mM chloral hydrate dissolved in saline. The motor was stepped to force 10 μl pulses of liquid out of the electrode tip, which was positioned as close as possible to the tip of the recording electrode.

Recording and stimulation techniques may be found in the methods sections of chapters 1 and 2.
RESULTS

The effects of the anaesthetics on resting and receptor potentials.

Upon addition of 60 mM chloral hydrate or 0.8 mM octanol to the perfusate, photoreceptors rapidly depolarized towards $V_{\text{max}}$ - the maximum response of the cell to light (Fig. 1). The resting potential of the cell came close to, or equaled, but never exceeded $V_{\text{max}}$ even when using larger doses of anaesthetic. Receptor potentials in response to brief flashes decreased in amplitude as the cell depolarized, but were never seen to reverse. Some cells underwent an initial peak depolarization followed by a fall away from $V_{\text{max}}$, the cell remaining insensitive to light throughout (Fig. 1). Upon the removal of the anaesthetic from the perfusate, cells repolarized and the resting potential returned to within 5 mV of its original level.

The time-courses of receptor potentials were not consistently altered by octanol or by doses of up to 60 mM chloral hydrate (Fig. 2). Larger doses of chloral hydrate (60-100 mM) induced a 'tail' on the falling phase of receptor potentials elicited as the cell depolarized, approximately doubling their decay time. Doses of less than 60 mM chloral hydrate produced depolarizations that were roughly proportional to the concentration applied. The lowest concentration of chloral hydrate to consistently depolarize photoreceptors was 10 mM.

100 mM butanol reversibly depolarized photoreceptors by 20-40 mV without change in the time course of their receptor potentials. Larger doses of butanol could not be applied due to its insolubility in saline.

Keeping the preparation in darkness, without stimulation, appeared to have no effect on the rate at which photoreceptors depolarized during the application of anaesthetic.
Figure 4.1.

The behaviour of a photoreceptor treated successively with chloral hydrate and with octanol. Black bars represent the amplitude of receptor potentials in response to 10ms flashes. The bottom of each bar represents the cell's potential in the dark relative to its resting potential at the beginning of the experiment.

Figure 4.2.

Receptor potentials recorded during treatment with 60 mM chloral hydrate. (a) Before treatment began; (b) 60s; (c) 150s; (d) 210s after the introduction of chloral hydrate. (e) shows a response recorded 7 min after perfusion with chloral hydrate had ceased. The bottom traces show the time of stimulation by a flash of intensity $10^{14}$ photons cm$^{-2}$ s$^{-1}$.

Figure 4.3.

The initial response of a photoreceptor to a pulse of 100 mM chloral hydrate delivered via a microsyringe.
The measured time-course of the anaesthetic effects described above were limited by the half-time of filling of the perfusion chamber (approx. 20s). A crude measure of the true latency of a cell's response to chloral hydrate was obtained using a microsyringe positioned above the recording site (see Methods). The latency of the depolarization following a 10 ul pulse of 100 mM chloral hydrate in saline was less than one second (fig. 3). The depolarization was accompanied by a characteristic increase in noise. Recovery of a cell's potential was usually complete within 90s of the delivery of the pulse of chloral hydrate.

**Anaesthetic-induced voltage noise**

Voltage noise recorded from photoreceptors in the dark had a variance, $E^2$, of 0.006 mV (mean of 12 cells; SE=0.002). A dark-adapted photoreceptor depolarizing in darkness during perfusion with salines containing chloral hydrate, octanol, or butanol exhibited increased low frequency voltage noise. As a cell depolarized, the amplitude of the membrane voltage noise rose, reached a maximum at a depolarization ($V_{\text{peak}}$) of 10-20 mV and then declined, disappearing if the cell were allowed to depolarize to $V_{\text{max}}$ before the chloral hydrate was removed. On removal of chloral hydrate, this process was reversed (fig. 4).

Samples of the noise recorded during the slow wash-out of chloral-hydrate from the perfusion saline were digitized. The difference between the noise variance during and after the action of chloral hydrate was plotted against the mean depolarization, $V$, of a photoreceptor during the gradual removal of anaesthetic (Fig. 5). As in chapter 2, on page 43, the relationship between the noise variance and the depolarization is also shown after correcting for non-linear summation (Katz and Miledi, 1972). A linear plot of the corrected
Figure 4.4.

(a) -(c): Voltage noise, recorded at the depolarizations shown during the gradual removal of 40 mM chloral hydrate from the perfusate. The final stages of drug action ((e) and (f)) and the response to subsequent weak illumination (h) are also shown.

Figure 4.5.

Voltage noise variance (increase from final value) plotted against the depolarization of a photoreceptor during the gradual removal of 50 mM chloral hydrate from the perfusing saline. Circles show raw data, triangles are data corrected for non-linear summation (see text). $V_D$ was set at 55 mV. The cell's potential after recovery was 1.5 mV hyperpolarized relative to that before chloral hydrate was applied and the voltage noise variance was unchanged at 0.006 mV. The variance was calculated from 2s records.
values is obtained for depolarizations of <15 mV, assuming, as in chapter, that the driving force, $V_d$, is equal to 4 times the depolarization at which noise is maximal ($V_{\text{peak}}$).

The value of $V_{\text{peak}}$ for 9 cells, $10.5 \text{ mV} \pm 1 \text{ mV (SE)}$, means that the value of $V_d$, 42 mV, lies in the range of maximum depolarizations obtainable using large amounts of chloral hydrate.

Nine cells had a mean event size of $36 \mu \text{V} \pm 8 \mu \text{V (SE)}$. Assuming a driving force of 60 mV and a cell input resistance of 15 MΩ (see chapter 1), a voltage event of $36 \mu \text{V}$ would arise from a conductance event of amplitude 40 pS.

**Effects of replacement of perfusate sodium**

Complete replacement of the perfusate sodium with choline reversibly reduces the amplitude of the noise and the depolarization that accompanies the steady illumination of a photoreceptor (chapter 1). This effect on the response to illumination was compared with that upon the voltage noise induced by chloral hydrate. After 120s perfusion with sodium-free saline, the voltage noise during steady depolarization by 30 mM chloral hydrate was reversibly reduced in r.m.s. amplitude to $29\% \pm 7\%$ of its original value, while the mean depolarization dropped from 8.5 mV to 4.7 mV (Fig. 6). Comparison with the results in chapter 2 shows that the effects of sodium replacement on the response to chloral hydrate are similar to those on the response to steady illumination.

**Experiments using double-barrelled electrodes**

1) Chloral hydrate reduces the cell input resistance.

The input resistance of cells fell during steady depolarization
Figure 4.6a

The reduction in amplitude of the depolarization and voltage noise induced by chloral hydrate during the complete replacement of perfusate sodium by choline. The recordings were made: (a) before treatment with chloral hydrate; (b) after 3 min treatment with 30 mM chloral hydrate; (c) 2 min after reverting to sodium ringer; (e) after subsequent removal of chloral hydrate.

Figure 4.6b.

The mean depolarization (dashed line) and relative r.m.s. chloral hydrate-induced voltage noise (solid line) recorded from 6 cells treated with 30 mM chloral hydrate and during sodium replacement with choline. Bars indicate standard errors.
induced by 40 mM chloral hydrate. Fig. 7 shows that the resistance during the depolarization of a cell is reduced by two factors. Firstly, the rectification properties of the membrane (chapter 1) and, secondly, a shunting, parallel resistance which can be estimated by comparing the resistance of the receptor before and during chloral hydrate treatment in the linear portion of the V-I curve, when the cell is hyperpolarized by current injection. This parallel resistance reduces the input resistance of the cell in this voltage range by 20-40% and it may reflect the channels that the analysis of voltage noise suggests are opening and closing during treatment with chloral hydrate. The magnitude of the shunting pathway adequately explains the depolarization of the cell (approx. 20% $V_{\text{max}}$) if we assume a driving potential equal to $V_{\text{max}}$ behind ion movements in the shunting pathway.

2) Double electrode experiments on voltage noise.

Experiments were performed to investigate the relationship between chloral hydrate-induced noise amplitude and the membrane potential during current injection. Fig. 8 summarizes the results. The r.m.s values of voltage noise are linearly dependent on the imposed membrane potential during 5s injections of current. The voltage noise in the absence of illumination or chloral hydrate was not significantly affected by the currents used. The extrapolated null potential for the chloral hydrate-induced noise was 64 mV $\pm$ 6 mV (SE) (n=4) above the cells' natural resting potential in darkness and for the noise induced by light it was 46 mV $\pm$ 2 mV. Because electrode coupling was only controlled to within 10% of a cell's input resistance, these values must be taken as upper estimates.
Figure 4.7.

The V-I curve of a photoreceptor before treatment with 40 mM chloral hydrate (filled squares) when depolarized 11 mV by chloral hydrate (triangles) and after recovery from chloral hydrate (open circles).

Figure 4.8.

The relative r.m.s. voltage noise, $\frac{\sigma V}{\sigma V_0}$, plotted against the potential imposed by 5s injections of current. (a) cell depolarized 14 mV by 40 mM chloral hydrate. (b) the same cell depolarized by steady illumination after recovery from drug. The potentials shown are relative to those at the beginning of the experiment.
Potentials (mV)

-4 -3 -2 -1 1 2 3 nA

-120 -100 -80 -60 -40 -20 0 20 40 60

-3nA 3nA

Potential (mV)

-20 0 +20 +40 +60

2 \sqrt{\sigma / \sigma_u}

-3nA 3nA

Potential (mV)

-40 -20 0 +20 +40
Return of a photoreceptors potential to its value before treatment with chloral hydrate thus increases rather than decreases the induced voltage noise. Conversely, an experiment in which a cell's membrane potential was held constant by applying increasing hyperpolarizing current showed the usual increase in voltage noise. Thus the increase in voltage noise is not a result of depolarization by the anaesthetic.

**Power spectral analysis of the voltage noise**

The power spectrum of voltage noise recorded from a dark-adapted photoreceptor in darkness (which includes amplifier and electrode noise) falls as \(1/\text{frequency, } f\), between 4Hz and 180 Hz. (fig. 9). This \(1/f\) relationship is typical of voltage noise recorded from high-resistance glass microelectrodes. (De Felice&Firth, 1970) and from 'resting' cell membranes (review: Neher and Stevens, 1977). During a depolarization of 12 mV induced by treatment with chloral hydrate, low frequency voltage noise increased and the power spectrum changed shape. The difference power spectrum between spectra recorded before and during chloral hydrate treatment (fig. 9) is flat between 1 and 20Hz. Above 20Hz it declines as \(1/f^2\) to \(1/f^3\), the steepness of roll-off varying from cell to cell. When all of the chloral hydrate was washed out of the perfusing saline, the power spectrum returns to the shape and amplitude that it had before drug treatment (fig. 9)

While the membrane potential recovered during the gradual removal of chloral hydrate, the difference spectrum of the induced noise did not change in shape, but it shifted to lower frequencies. This is to be expected if the electrical time constant of the cell largely determines the power spectral shape. As the cell recovers, its membrane resistance, and hence its time constant will increase and the noise spectrum will therefore be shifted to lower frequencies.
Figure 4.9.

The difference power spectrum (squares) of the noise increase caused by 40 mM chloral hydrate at a depolarization of 12 mV. The other spectra were recorded before (triangles) and after (circles) chloral hydrate treatment. 22°C.

Figure 4.10.

Power spectra of voltage noise (difference spectra) created by illumination (continuous lines) or by chloral hydrate (dotted lines) in the same cell. The spectra were recorded when the cell was depolarized 12 mV and 22 mV by chloral hydrate or by illumination following the recovery of the cell from chloral hydrate. The light intensities were $7 \times 10^{11}$ photons.cm$^{-2}$.s$^{-1}$ and $3 \times 10^{13}$ photons.cm$^{-2}$.s$^{-1}$; wavelength 560nm. The variances of the noise were 0.7 mV (light, 22 mV); 0.4 mV (drug, 22 mV); 1.9 mV (light, 12 mV); 0.5 mV (drug, 12 mV). 22°C. 10s samples.
Figure 10 compares the noise produced by chloral hydrate with that produced during subsequent illumination. As with the noise induced by azide (chapter 2) and fluoride (chapter 3), the chloral hydrate-induced noise has a power spectral shape that mimics the spectrum of light-induced noise at a large depolarization but which rolls-off less steeply when the two are compared at a lower depolarization. The chloral hydrate induced noise also has a very much smaller amplitude when compared to light-induced noise at small depolarizations due to the differences in their event sizes (35 \mu V compared with 500 \mu V to 5 mV respectively). The similarity in shape develops, as in previous chapters, as the light intensity is increased beyond $10^{12}$ photons.cm$^{-2}$.s$^{-1}$. Over the same intensity range, the variance of light-induced noise decreases to approach that of chloral hydrate-induced noise recorded at the same depolarization. However, in contrast to fluoride-induced noise, the standard deviations of light and chloral hydrate-induced noise do not become equal. At a mean depolarization of 14 mV seven cells exhibited a ratio between the r.m.s. amplitudes of light-induced noise to chloral hydrate-induced noise of $1.58 \pm 0.38$.

The effects of fluoride-treatment on the response to chloral hydrate

Many of the characteristics of the noise induced by chloral hydrate are similar to that seen following fluoride treatment (chapter 3). The major differences are that chloral hydrate acts very much more rapidly than fluoride or azide in darkness and that illumination has no apparent effect on the time-course of the depolarization. Following the model of chapter 3 (Fig. 3.10) we thought that it was possible that chloral hydrate directly activates the enzyme that releases "active transmitter", so as to produce a prolonged burst of
Figure 4.11.

Upper left: High-gain, a. c coupled traces and low-gain, d. c. coupled traces of the response of a cell to a pulse of chloral hydrate delivered through a microsyringe.

Upper right: Another pulse of chloral hydrate, but delivered to the same cell after 10 mM NaF had been applied for 160s and then washed out for 20s.

Lower left: The noise recorded before the syringe pulse (upper) and the chloral-hydrate-induced noise (lower) are displayed on a faster time-scale.

Lower right: The voltage noise recorded before the syringe pulse (upper) and during the chloral hydrate /fluoride-induced afterpotential are displayed on a faster time base.
uncorrelated channel openings. According to the model of fig. 3.10, fluoride acts by blocking the inactivation of this enzyme or by blocking the inactivation of the transmitter. If this is the case then, following treatment with fluoride, chloral hydrate should, like light, be able to initiate a sustained noisy depolarization. This prediction was tested by applying a pulse of chloral hydrate to a photoreceptor through a microsyringe before and after the preparation was treated for 2-3mins with 10 mM NaF. Following fluoride treatment the response to the chloral hydrate is larger and a sustained, noisy depolarization follows (Fig. 11).
DISCUSSION

Summary of results

The simple anaesthetic molecules investigated have a complex effect on the electrophysiology of retinula cells. Analysis of the voltage noise, accompanying the rapid depolarization induced by these compounds, suggests that it may be caused by the random summation of voltage events of amplitude 35 μV. The peak depolarization induced by large doses of anaesthetics, the effects of a change in membrane potential imposed by current injection and the relationship between the noise variance and the chloral hydrate-induced depolarization all indicate that the noise may be caused by channels through which ions permeate with a driving force between 40 and 60 mV above the cell's resting potential. This range is close to $V_{\text{max}}$ and the driving force predicted for the response to saturating illumination (chapter 1). Ion replacement experiments suggest that the channels opened are permeable by sodium.

The effects of a pulse of chloral hydrate are usually rapidly reversible. However, following the application of NaF, the noisy depolarization induced by anaesthetics is sustained as an irreversible afterpotential.

The nature of the elementary events

Neutral anaesthetics inhibit or facilitate cation movements across a variety of artificial and natural membranes (Armstrong and Binstock, 1964; Gage et al., 1975; Gutknecht and Tosteson 1970; Knutson, 1961). Such a rapid, large and reversible depolarization of a membrane, possibly because of an increased sodium conductance, has not, however, been described by these authors. Rather than causing a general leakiness to cations, the voltage noise suggests that
ionic pathways similar to those opened following fluoride treatment are being activated in the cell's membranes. The characteristics of the noise induced by chloral hydrate have a great deal in common with the noise seen following fluoride treatment (chapter 3). The rapidity with which chloral hydrate acts, and the results of fig. 11, suggest that chloral hydrate is able to directly activate the same stage in transduction that appears to be locked into an active state by fluoride. Thus, to extend the model of Fig. 3.10, it is possible that chloral hydrate causes the spontaneous release into the cytoplasm of 'internal transmitter'. However, it must be pointed out that the amplitude of the chloral hydrate-induced noise is, on average, less than that recorded from illuminated photoreceptors even when the induced depolarizations are great (page 33). This contrasts with the equivalence of fluoride-induced noise and noise from a light-adapted cell (chapter 3). It is possible that chloral hydrate has other actions, apart from opening some of the light-activated channels, that would account for this discrepancy. An ability to depolarize photoreceptors without an increase in noise is one possibility.

Recently Shaw et al. (1978) have also reported modifications of transduction in barnacle photoreceptors, following treatment with alcohols. A sustained, reversible depolarization follows light flashes delivered to a photoreceptor in the presence of alcohols. It remains to be seen whether this depolarization can be related to an increased conductance of the receptor's membrane.

Whatever the cause of the phenomena presented in this paper, further investigation of activators and inhibitors of transduction on this preparation may have to take account of the possible 'anaesthetic' effects of lipid-soluble compounds, particularly when they are applied to the saline in high concentrations in an attempt to
modify processes within the photoreceptor.
CHAPTER FIVE:

MODELLING OF THE IMPULSE-RESPONSE OF LOCUST PHOTORECEPTORS
SUMMARY

1) The response of locust photoreceptors to brief weak flashes (the 'impulse-response') is examined using intact and superfused eyes.

2) Attempts to fit the impulse-response with models which involve cascades of first-order exponential delays required too many stages, with too great a variation in their rate constants from cell to cell, for them to be physically plausible.

3) The reduction in the time-scale and the sensitivity of the impulse-response that is brought about by light-adaptation requires that light-adaptation affects the latency and the gain of the transducer independently.

4) Both light-adaptation and changes in temperature induce a simple change in the time-scale of the impulse-response. The $Q_{10}$ of the temperature effect is 2.

5) The impulse response follows the lognormal curve. A simple model of visual transduction is developed to account for the lognormal shape, and the implications of the model for the non-linear response of the photoreceptor to very bright flashes are discussed.
INTRODUCTION

An explanation for the long delay between the absorption of a photon and the electrical response of a photoreceptor has posed a considerable problem for theoretical models of phototransduction. Fuortes and Hodgkin (1964) sought to explain the delay as being due to a cascade of amplifying elements each with a limited high-frequency response. The cascade (hereafter called the F-H model) was modelled as a series of $n$ R-C filters with intervening, isolating amplifiers (fig. la). The response of the system to a brief flash, of intensity $I$, over a small linear range was a gamma function:

$$V(t) = \frac{I \Delta t A t^{n-1}}{(n-1)!} \cdot \exp(t/R.C) \quad (1)$$

where $\tau = R \cdot C$ is the time constant of each filter stage and $\Delta t$ is the flash duration. $A$ is a constant which does not involve $R$.

A powerful attraction of the model is that variation in a single parameter, $R$, modifies the time-to-peak, $t_p$, and the sensitivity of the response, $S$, simultaneously by the relations:

$$t_p = (n-1)R.C \quad (2)$$

and

$$S = A' \cdot (t_p)^{n-1} \quad (3)$$

here $A'$ is another constant which depends on $A$ and $n$. 
(a) The Fuortes and Hodgkin (1964) model of visual transduction. The model consists of a cascade of R-C low-pass filters, separated by isolating elements with infinite input impedance and a mutual conductance equal to $u$.

(b) Borsellino, Fuortes and Smith (1965) produced a biochemical correlate of the F-H model. The enzyme precursors, $E_1\ldots E_n$, are converted to active forms, $E_1^{*}\ldots E_n^{*}$ with rate $\lambda$ before they decay to inactive states with rate $u$. Once they are active, the enzymes are then able to activate the next enzyme precursor in the chain until a final product $E_n$ is formed, which generates an electrical potential difference across the photoreceptor's membranes.

(c) The Baylor-Hodgkin-Lamb (1974) model, developed as a basis for an investigation of the response of vertebrate retinal cones, has 5 non-amplifying stages with rates $6\alpha$, $5\alpha$, $4\alpha$ etc. These stages produce an active transmitter, $Z$, which then decays through a final stage which has a rate $\beta\alpha$. In the simplest case that they considered, $\beta$ was equal to 1.
Light adaptation could be shown to reduce the time-scale and sensitivity of the response in accordance with equation (3) and it was therefore proposed that feedback from a late stage in the process was able to reduce the resistances, $R$, of the filters.

Borsellino et al. (1965) proposed a biochemical correlate of the F-H model (hereafter called the 'B-F-S model'). The cascade of amplifiers was replaced by a biochemical cascade of enzymes producing a final product that modulated the photoreceptor's electrical potential (fig. 1b). The reduction in time-scale and sensitivity associated with light adaptation could now be described as an increase in the rate at which enzymes were removed from the cascade by increasing the time-constant, $\mu$, of the reactions by which enzymes decay to inactive states. Borsellino and Fuortes (1968) produced a stochastic approximation to the enzyme cascade and concluded that some amplification was required to account for the stochastic properties of the latency of the response of Limulus photoreceptors to weak flashes.

Following this early work, it has become clear that many photoreceptors respond to a single photon with a large 'bump' which arises with considerable variation in latency and has a variable amplitude and time-course. The responses modelled by Fuortes and Hodgkin constitute the mean response of a system with considerable stochastic variation. Subsequent modelling of the response of Limulus photoreceptors has subdivided the response to light into an amplifying 'bump process' and a non-amplifying 'latency process'. The bump process of Limulus ventral eye photoreceptors has been elegantly modeled with a gamma function with $n=3$ by Wong (1978) while Wong et al (1980) have, in addition, described the latency process in the dark- and light- adapted states.
The first order reaction cascade has remained a popular model for the latency and bump processes. Following a suggestion by Wong et al (1980), Tiedge (1981) has formalized the processes proposed by Wong et al (1980) as a chain of 7 non-amplifying first-order chain reactions followed by three cascaded amplifying reactions, also first order. If the shape and amplitude of a bump is independent of its latency, then the gamma distribution \( n=3 \) used by Wong et al (1980) to describe the 'average bump' will convolve with the gamma distribution \( n=7 \) describing the latency chain to produce an impulse response identical to that of Fuortes and Hodgkin (a gamma distribution with \( n=10 \)). The chain reaction model has also been applied to vertebrate cones by Baylor et al (1974), using a chain of \( n-1 \) non-amplifying stages with rates equal to \( n\alpha, (n-1)\alpha, (n-2)\alpha \) etc. and one decay stage with a different rate, equal to \( \alpha\beta \) (fig. 1c; hereafter called the B-H-L model). As with previous chain-reaction models, light-adaptation was modelled by an increase in the rate of the decay stage, through an increase in \( \beta \).

For the simplest case, when \( \beta = 1 \), the B-H-L model predicts that the impulse-response will have the following form.

\[
V(t) = n.c.I.\Delta t.exp(-\alpha t).(1-exp(-\alpha t))^{n-1}
\]

(4)

(\text{where } c \text{ is a constant})

The B-H-L model has been applied to the response of an invertebrate photoreceptor (cephalopod) by Duncan and Pysent (1979). For cephalopod photoreceptors \( n = 10 \) was found to produce the best fit, while \( n = 6 \) was found by Baylor et al (1974) to produce the best fit to the responses of vertebrate cone cells.

Responses from a wide variety of invertebrate photoreceptors have now been recorded intracellularly, particularly those of insects. A systematic study of their transduction kinetics is long overdue. The purpose of such a comparative study would be to apply quantitative concepts and models of transduction in order to find a unifying theory of phototransduction. Several questions might be answered by a
comparative study. Can the response of all invertebrate photoreceptors be described by chain reaction models? Why do some receptors produce large bumps while others do not and is this difference related to differences in a parameter in a model of transduction (e.g. the number of amplifying stages)? Can new kinetic phenomena be discovered to shed light on transduction?

The comparative approach is potentially powerful, but the results obtained so far have lacked consistency. Studies by Zettler (1969), Pinter (1972), Smola and Germperlein (1972), Leutscher-Hazelhoff (1975), French and Jarvilheto (1978) and French (1980) have provided no clear picture of the kinetics of phototransduction in insect photoreceptors. There is disagreement as to whether cascades of first-order reactions can model the frequency response of the photoreceptors and as to whether changes in gain and time scale with light adaptation are linked by a common mechanism. This confusion probably arises from the methods used to measure the frequency response of the photoreceptors. The above authors have used either sine-wave or white-noise modulation of light to produce Bode plots of the gain and phase shift associated with phototransduction. Both techniques involve a modulation of a signal about a mean light intensity that produces a mean depolarization of several mV, so that the preparations are never in a truly dark adapted state. In addition, the time taken to obtain accurate data using sine wave modulation introduces uncertainties due to the instabilities associated with recording from such small cells as insect photoreceptors.

No clear conclusion has therefore been reached as to the differences between the impulse-response of insect and Limulus photoreceptors and no small set of parameters has yet been extracted from the data to describe the response of insect cells - a necessary
first step in a quantitative comparative study.

The following chapter describes an analysis of the impulse-response of a locust photoreceptor, mainly in its linear range. The impulse response contains equivalent information to the frequency response of a photoreceptor which has been shown to be linear for small modulations of light intensity (Pinter, 1972; Howard, in the press) and it is easily and quickly measured. A direct comparison with the predictions of the F-H model and other models of transduction can be made and their success in describing the response ascertained. We show that the impulse response of a locust photoreceptor cannot be adequately modelled with a chain of first order reactions, with or without amplification at some stages. We also show that the effects of light adaptation cannot be explained solely by an increase in the rate of decay of an intermediate in such a chain. We propose a simple empirical equation—the lognormal equation—for the impulse-response of an insect photoreceptor and we show how such an equation can be generated from a system with a biochemical threshold using a minimum of assumptions.

The chapter takes the form of a reprint of a brief paper (Payne and Howard, 1981) in which the observations and arguments are summarized, followed by some further experimental observations and a discussion.
Response of an insect photoreceptor: a simple log-normal model

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Photoreceptors respond to light with a graded depolarization or hyperpolarization of the cell membrane. The transduction of light energy to an electrical event, though much studied, remains an outstanding problem in visual research. Previous transduction models\(^1\), involving cascades of chemical reactions, do not fit the responses of insect photoreceptors\(^2\). It has been proposed that the absorption of light causes the release of an alternative model. We have developed here a simple two-parameter model of transduction based on the assumption that membrane channels are activated when the concentration of internal transmitter reaches a threshold level\(^4\). The theory predicts that the impulse–response function follows the log-normal curve—this model fits the response of the locust compound eye photoreceptor over the 10-fold range of time scale produced by altering the temperature or the state of light adaptation of the cell.

Photoreceptors in the compound eye of Locusta migratoria generate a variable number of superimposed, depolarizing voltage events when stimulated with a brief, weak flash of light\(^5\). Each event or ‘bump’ represents an effective quantal absorption of light\(^4\). The average of a large number of such responses accurately defines the impulse–response function of the receptor. The amplitude of this response increases linearly with increasing flash intensity when the responses have an amplitude of <$0.5$ mV and in this linear range, the Fourier transformation of the impulse–response function corresponds to the temporal frequency response of the receptor\(^6\).

Using glass micropipettes, recordings of electrical potential were made from photoreceptors in an intact eye\(^7\) and a superfused retina\(^8\). For the superfused retina we averaged responses from dark-adapted photoreceptors to 1-ms flashes from a green light-emitting diode (Siemens LD57C) at temperatures of the surrounding perfusion fluid between 33 and 17 °C. When recording from photoreceptors in an intact eye, a 1-ms test flash from a point light source (also a LED) could be superimposed on the electrical potential of the ommatidium containing the impaled cell.

We attempted to fit our impulse–response functions to the models of Fuortes and Hodgkin\(^9\) and Baylor et al.\(^10\). These models are based on the time course of the concentration of the penultimate product of a series of $n$ reactions which proceed sequentially\(^9\) or independently\(^10\). Like Borsellino et al.\(^11\), we found that the Fuortes and Hodgkin model predicted a response that was too symmetrical about the peak and it also required a range of values of $n$ between 13 and 20 to fit the data from all our cells. The model of Baylor et al. fitted the response shape adequately, but required values of $n$ between 20 and 48. The large values for the number of steps required in the reaction chains of these models and the variability from cell to cell made us doubt their physical applicability. We therefore searched for an alternative model.

We chose to fit our impulse–response functions to the two-parameter log-normal curve (Fig. 1a)\(^\text{a}^1\)

\[
V(t) = s I \Delta t \cdot t^{-1} \cdot \exp\left[-(\log(t/\mu))^2/2\sigma^2\right] \tag{1}
\]

where $I$ is the intensity of the flash of duration $\Delta t$ delivered at $t = 0$ and $s$ is the sensitivity of the cell, $\sigma$ is the standard deviation of the transformed gaussian curve.

An adapting background of intensity $3 \times 10^4$ photons per facet per s reduced the value of $\mu$ for responses from cells in the intact eye from $60 \pm 18$ ms ($n = 6$) in the dark-adapted state to $24 \pm 2$ ms when light adapted. The corresponding values of $\sigma$, 0.30 ± 0.01 and 0.32 ± 0.02, showed no significant change (for example, see Fig. 1a). A large decrease in the sensitivity of the response also occurred during light adaptation as noted by others\(^12\). Lowering the temperature of the superfused preparation from 33 to 21 °C resulted in an increase in $\mu$ from $70 \pm 22$ ms ($n = 7$) to 160 ± 14 ms. A marginal increase in $\sigma$ from 0.28 ± 0.02 to 0.32 ± 0.02 also occurred (for example, Fig. 1b).

\[
\log_{10}[\mu \exp(-\sigma^2)]
\]

**Fig. 1** a, Impulse–response functions recorded from a photoreceptor in an intact eye when dark adapted and at two light-adapted states. One hundred presentations of a 1-ms light flash were averaged in each case. The intensities of the flashes and superimposed adapting light were: 14 photons per facet per flash (dark adapted), 42 photons per facet per flash superimposed on an adapting light of $1.3 \times 10^4$ photons per flash per s and $4.2 \times 10^2$ photons per facet per flash superimposed on an adapting light of $1.3 \times 10^4$ photons per facet per s, wavelength $560$ nm. Circles show the fits to the data using equation (1) and the following parameters: DA: $\sigma = 0.21$, $\mu = 74$ ms; LA1: $\sigma = 0.27$, $\mu = 41$ ms; LA2: $\sigma = 0.31$, $\mu = 23$ ms. The amplitudes of the responses were 1.5 (DA), 1.2 (LA1) and 2.3 (LA2) mV. Inset, the impulse–response function in the dark-adapted state is replotted on a logarithmic time base. Equation (1) (circles) predicts that a gaussian curve will result, having mean $\mu$ and standard deviation $\sigma$. b, Impulse–response functions of a photoreceptor in a perfused eye at three temperatures. Circles show the fit to the data of equation (1) using the following parameter values: 17 °C: $\sigma = 0.305$, $\mu = 394$ ms; 22 °C: $\sigma = 0.294$, $\mu = 142$ ms; 33 °C: $\sigma = 0.247$, $\mu = 75$ ms. The responses shown are averages of 100 presentations of a 1-ms flash of intensity $1 \times 10^{12}$ photons cm$^{-2}$ s$^{-1}$ and wavelength $560$ nm. The amplitudes of the responses, which have been scaled to unit height, were 3.1 (17 °C), 3.9 (21 °C) and 3.8 (33 °C) mV.
Temperature and light adaptation therefore significantly affect only one temporal parameter in our model \( (\mu_t) \), corresponding to a change in the time scale of the response. We propose that this parameter represents the rate constant of a single transduction process, being sensitive to both light adaptation and temperature.

The log-normal distribution has a general application to the analysis of biological data with a large variance\(^1\). In particular, Gaddum\(^2\) and Bliss\(^3\) have used the distribution to describe the variability, from subject to subject, in the critical dose of a drug that will just cause a reaction. We propose an analogous physical model of phototransduction. The model assumes that, following the absorption of light, the strength of a chemical signal increases. The broad impulse–response arises from a scatter in the detection of threshold levels of this signal that trigger the activation of depolarizing ionic channels in the photoreceptive membrane.

Specifically, we assume that: (1) a photoisomerization opens several ionic channels in the vicinity of the activated rhodopsin molecule through the action of an internal transmitter. A given channel opens for a brief time when the concentration of transmitter exceeds a threshold value, \( \alpha \). (2) The threshold value, \( \alpha \), is normally distributed from channel to channel. (3) The time course of local transmitter concentration following a photoisomerization is a smooth, monotonic function rising from zero at the instant of the stimulus to a positive value, before declining. Its time course is governed by a single time constant, \( k \), so that the transmitter concentration is \( y = f(kt) \), where \( t \) is the time elapsed after the light flash. (4) The time constant, \( k \), is chosen so as to minimize the temporal width of the voltage generated for a given time to peak of the response \( t_p \).

A direct consequence of assumptions (3) and (4) is that the transmitter concentration, \( f(kt) \), increases almost logarithmically with respect to time in the neighbourhood of \( t_p \). This is because, by assumption (4), \( k \) is chosen so as to maximize the rate of increase in transmitter concentration, \( df(kt)/dt \), at \( t = t_p \); so that

\[
\frac{df(kt)}{dt} = 0 \quad \text{at} \quad t = t_p
\]  

However, from the identity

\[
\frac{df(kt)}{dt} = \frac{d}{\log t} \left( \frac{df(kt)}{d \log t} \right) = k \frac{df(kt)}{d \log t}
\]  

it follows that for our optimized system

\[
\frac{df(kt)}{d \log t} = k \frac{df(kt)}{d \log t} = 0 \quad \text{at} \quad t = t_p
\]  

Integrating equation (4) twice with respect to \( \log (t) \) yields \( f(kt) = a \log (t) + b \) in the neighbourhood of \( t_p \). The impulse–response function represents the sum of the voltages generated by all the channels that open following a light flash. This population of channels has, by assumption (2), thresholds to transmitter concentration that are normally distributed. If the concentration of transmitter rises logarithmically with time it follows that the temporal frequency of opening of channels will be log-normal so that, if channel opening times are brief, the voltage generated will follow equation (1). The model is illustrated in Fig. 2.

Our model describes the photoreceptor response as the result of a variable threshold associated with a process that is logarithmically related to time. Of the two temporal parameters only \( \mu_t \), which describes the rate of release of the internal transmitter, is significantly affected by temperature or light adaptation. We intend to continue our investigations by examining the response of ‘faster’ insect eyes, such as those of the fly and dragonfly. If equation (1) can be applied to other photoreceptors, then the two parameters, \( \mu_t \) and \( \alpha \), will provide a simple method of comparing their temporal frequency response, similar to the parameters describing the approximately gaussian angular sensitivity curves of photoreceptors\(^4\).

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Further observations on the impulse response.

1) The fitting of first order reaction chains

A surprising result of our investigation was the variability, from cell to cell, in the form of the impulse-response. The chain reaction models of B-F-S, B-H-L, and Tiedge (1981) require that this variation in form be fitted by a variation in the number of stages in the chains (fig 2). Since the number of stages in phototransduction might reasonably be assumed to be constant for a given species, this variation in the models is clearly untenable. For a general first order chain the variations in form could be accounted for by arbitrarily varying the rate constants of the various stages. Thus the data of fig. 2a and b could be fitted by a chain of about 20 stages with time constants which varied from 15ms to 0.7ms in the case of 2a, but which were all equal to 3ms in the case of 2b. However, this degree of variation in the kinetics of each stage also seems to us to be unreasonable.

At least 20 stages would be required to fit all our data because this is the number required to fit the most symmetrical model - the F-H model.

The changes of gain and time-scale during light adaptation.

A plot of the sensitivity of the impulse-response (expressed as peak mV per photon) against the time-to-peak response at various background intensities (fig. 3; replotted from Howard (in press)) clearly shows that Eq. 3 (page 89) cannot hold if n=10 to 20. The plot shows substantial shifts in the time-to-peak, accompanied by only small changes in gain, when low background intensities are applied. This observation has been confirmed for fly retinula cells by Dubs (in the press). Eq. 3, however, only applies to models in which the time
Figure 5.2

(a) The impulse-response (solid line), recorded from the superfused retina, that required the **largest** value of the lognormal parameter, \( \sigma \), in order to produce a good fit (\( \sigma = 0.31 \)). The F-H model (dotted line) requires 12 stages, each with a time constant of 4 ms, to produce the best least squares fit. The B-H-L model (dashes) requires 23 stages, with \( 1/\alpha = 15 \text{ ms} \).

(b) The impulse-response (solid line) that required the **least** value of \( \sigma \) to produce a good fit (\( \sigma = 0.23 \)). The F-H model requires 20 stages each with a time constant of 3 ms. The B-H-L model requires 48 stages with \( 1/\alpha = 15 \text{ ms} \).

Both responses were recorded at 29°C.
2a

2b

Normalized Response vs. Time After Flash (ms)
constants for the decay of all the elements in the cascade are altered equally. Can a chain model such as that of B-H-L or Wong et al (1980), in which gain control occurs at the last stage(s) only, explain the result? This question may be answered by plotting impulse-responses obtained at various adaptation states, having scaled them according to their sensitivity relative to that of the dark-adapted state (Fig. 4a). The responses then represent the impulse response of a cell to a flash of the same intensity at different adaptation states. Fig. 4b shows exactly the latter data obtained by Dubs (in the press) from a fly retinula cell.

Models of reaction chains in which light-adaptation is reproduced solely by an increase in the rate of decay of a number of chain elements will predict that the response to a flash of a given intensity delivered to a light-adapted photoreceptor cannot exceed the response to the same flash delivered to a dark-adapted cell, although it may equal it at early times. This is clearly not the case in figs. 4a and b, in which the light adapted responses do exceed the dark adapted ones. To correctly reproduce this observation, an increase in the rate with which forward reactions occur in the chain is also required. Thus the B-F-S model requires an increase in $\lambda$ as well as $\mu$, while the B-H-L model requires that $\alpha$ as well as $\beta$ increase. The models of Wong et al require that the rate of the latency process increases as well as the rate of the decay of the enzymes in the bump process. Wong et al. (1980) report that this is indeed the case in Limulus lateral eye photoreceptors.

The $Q_{10}$ of the time-scale of the response.

The impulse responses reported on page 94 were measured at two or three temperatures only. The time taken to dark-adapt cells (20-30 mins), to perform the necessary averaging, and to return for control
The logarithm of the sensitivity, $S$, of the impulse-response (expressed as peak mV per photon), relative to the dark adapted sensitivity $S'$, is plotted as a function of the logarithm of the time-to-peak of the impulse-response, $T_p$, relative to its dark-adapted value, $T_{p'}$. Data from several cells are shown. The data were obtained from Howard (in the press).
Figure 5.4

(a) Impulse-responses, obtained from intact eyes by J. Howard, are shown in the dark-adapted state (DA) and in two light-adapted states (LA1, LA2). The amplitudes of LA1 and LA2 have been scaled according to the sensitivity of the response relative to the sensitivity of the dark adapted state (see text). The light intensities of the test flashes were 32 photons per facet per flash (DA), 80 photons per facet per flash (LA1) and 720 photons per facet per flash (LA2). The background intensities for LA1 and LA2 were 20 photons per facet per flash and 315 photons per facet per flash respectively. The amplitudes of the responses were 2.2 mV (DA), 3.9 mV (LA1) and 2.11 mV (LA2).

(b) The effect of an adapting, background light on the impulse-response of a fly photoreceptor. The background light depolarized the receptor by 5 mV. The responses in the dark-adapted state (DA) and when light-adapted (LA) were both produced by very brief flashes with intensity equivalent to 5 effective photons per receptor. The figure has been re-drawn from Dubs (in the press).
**Figure 4a**

- **NORMALIZED RESPONSE**
- **TIME AFTER FLASH (ms)**
- Curves labeled DA, LA1, LA2

**Figure 4b**

- **mV**
- **ms after flash**
- Curves labeled LA, DA
runs at 33°C precluded the possibility of investigating the responses at more than one or two temperatures before the penetration of the cell became unstable. The limited results obtained suggested that the parameter $\mu$, governing the time scale of the response had a $Q_{10}$ of 2, while the $Q_{10}$ of $\sigma$ was only 0.14.

Responses to brief (5ms) bright flashes required less averaging and no time for dark adaptation, so that a wider range of temperatures could be investigated. The peak amplitudes of the responses to these flashes were 20-35 mV, well out of the linear range of a cell's response. As with the linear response, the effect of lowering the temperature could be explained as a change in the time-scale of the response with very little change in its form. Fig. 5 shows the responses of a cell at 4 temperatures plotted using a linear time axis (5a) and using a logarithmic time axis (5b) after normalization of the peak response. The responses are seen to be shifted along the log-time axis with little change in shape, so that only their time-scales are altered. For fig. 6, responses of 8 cells, at two temperatures, were averaged, plotted on a logarithmic time axis and their position along the log-time axis was adjusted so that their maxima coincided. The similarity of the two curves after this process again illustrates the point that altering the temperature simply causes a change in the response time-scale.

Fig. 7 is an Arrhenius plot of the times-to-peak of 4 cells. The times-to-peak of 8 cells were found to have an average $Q_{10}$ of 2 between 33°C and 17°C. $Q_{10}$ 's of 1.7 for fly (French and Jarvilheto, 1978) and 3.2 for Limulus (Borsellino, Fuortes and Smith, 1965) have been reported previously. Baylor et al (1974) report that the $Q_{10}$ of the time-to-peak of the linear response of vertebrate cones is 1.8.
Figure 5.5

(a) Receptor potentials recorded from the perfused eye in response to 2ms flashes of intensity $10^{14}$ photons.cm$^{-2}$.s$^{-1}$ at four different temperatures.

(b) The responses of 5.5 (a) are normalized to their peaks and plotted on a logarithmic time-scale, originating at the time of the stimulating light flash.

Figure 5.6

The responses of 8 cells to a bright 2ms flash at 2 temperatures (33°C and 17°C) were plotted, as in fig.5.5 (b), on a logarithmic time base. The responses were then shifted along the log-time axis so that their maxima coincided, and the responses at each temperature were averaged over the 8 cells.

Figure 5.7

An Arrhenius plot of the logarithm of the time-to-peak of large-amplitude (non-linear) responses of 4 cells as a function of the reciprocal temperature. The lines are the linear regression through the data from each cell.
Both the value for the $Q_{10}$ reported here and the invariance in the form of the impulse-response at different temperatures [also reported for *Limulus* lateral eye by Borsellino et al (1968)] are apparently at variance with the report of Wong et al (1980). The latter report that the latency process in *Limulus* lateral eye photoreceptors has a $Q_{10} \approx 5$, while the $Q_{10}$ of the bump time-scale is only 2.5. Measurement of the latencies and time-scale of *Locusta* bumps may provide an explanation for this discrepancy. However, preliminary observation of the latter, as well the invariance of the impulse-response, shape suggest that, for locust, both processes will have a similar $Q_{10}$ between 33°C and 17°C.
Discussion

Chain reaction models

First order chain reaction models involving one chain (B-F-S, B-H-L models), or when broken into independent latency and bump processes (Wong et al (1980), 1980; Tiedge, 1981), require too many stages, with too great a variability from cell to cell, to adequately model impulse-responses of locust photoreceptors. This means that a new delay mechanism, involving fewer elements, may be required.

To explain the effects of background illumination on the sensitivity and time scale of the impulse response, models with cascades of amplifying elements must be modified by increasing both the rate of decay of the amplifying particles (enzymes) and the rate of forward activation along the chain. For models which involve separate delay and amplification stages, this means that the delay and the amplification must be modified independently. Thus the tight coupling between time-course and sensitivity proposed by Fuortes and Hodgkin (1964) may not hold for insect photoreceptors. Wong et al. (1980) show, using noise analysis, that the rate of their latency process is increased by light adaptation, concurrently with a reduction in the gain and the duration of the bump process.

The lognormal model

The lognormal model presented in this chapter describes a biochemical mechanism by which a delay might be built into the system using very few elements. The model takes an extreme case, in that the whole impulse-response is described by only a single, highly non-linear stage. This results in an economy of parameters. However, modifications, probably involving more parameters, will be needed to describe the production of bumps, just as chain-reaction models have
been split into separate latency and bump processes.

**Local non-linearities in the lognormal model**

The lognormal model involves a threshold mechanism in the physical locality of the isomerized rhodopsin molecule. The transmitter is assumed to be localized to some small area around the isomerized rhodopsin molecule. One might call this area around a rhodopsin molecule, within which the transmitter reaches threshold concentration, a 'transduction unit'.

For light intensities of flashes which have a very low probability of activating neighbouring transduction units, the model predicts linear kinetics. However, for higher light intensities, two forms of non-linearity will develop:

a) The absorption of a photon simultaneously in two neighbouring transduction units may result in transmitter from one unit spilling over into the area of the other unit, thus reducing the time taken to open channels. This is the 'pre-bump' phenomenon of Hamdorf and Kirschfeld (1980). Responses to flashes of sufficient intensity to activate neighbouring transduction units will therefore display non-linearity at the earliest times in the response due to this decrease in the time taken to reach threshold. This is a necessary quality of models involving thresholds in which interaction between transduction units is allowed, but it is not a prediction of linear chain reaction models with feedback arising from a late stage. Linearity at early times has been demonstrated in *Limulus* lateral eye photoreceptors over 3 log units of light intensity (Borsellino and Fuortes, 1968). The work of Hamdorf and Kirschfeld (1980) suggests that the non-linearities due to 'prebumps' will begin to become significant at flash intensities of greater than $10^4$ effective
photons per receptor, when neighbouring microvilli are often activated by flashes. The linearity of the response of dark adapted receptors to flashes should therefore be re-examined at higher light intensities than those used by Borsellino and Fuortes. Alternatively, the light could be focused onto a small area of rhabdom using axial illumination of a cut, superfused retina.

b) The lognormal model requires that, once channels have been opened briefly, they remain in a closed, refractory state for as long as it takes for the local concentration of transmitter to decline below the threshold level. This assumption requires that a second photon hitting the same transduction site during this refractory period will be ineffective. The refractory period may also be experimentally detectable using localised stimuli. Certainly photons do compete for transduction units during brief flashes (Brown and Coles, 1979) but it is not known for how long the channels remain 'occupied'.

An investigation of the two non-linearities described above will provide a means of testing the model of phototransduction that we have proposed.

**Light adaptation and the lognormal model**

The non-linearity described above in section (a) would not be appreciable for the light-adapted test flashes used in the experiments of this chapter. The reduction in the time scale and sensitivity of the impulse-response must therefore be attributed to another process. We propose, as did Fuortes and Hodgkin, that feedback from a late stage in transduction, possibly involving a delayed release of calcium (Lisman and Brown, 1972), increases the rate at which transmitter is produced and also reduces the number of channels opened per photon.
(Wong, 1978). The reduction in sensitivity could be achieved by a reduction in the area of the transduction unit (Wong et al., 1980). In the light-adapted state, therefore, the local transmitter concentration rises faster, but reaches the threshold concentration within a smaller area of membrane.

In summary, we feel that the use of the lognormal equation of the impulse-response will prove to provide a set of easily interpretable parameters with which to quantify a comparative study of phototransduction. In addition, the data gathered already from the locust and fly has provided a stimulus for the search for a more satisfactory model of the delay inherent in phototransduction than is provided by models involving chains of first order reactions. We have presented quantitative and qualitative predictions obtained from one alternative, a model with a threshold.
GENERAL DISCUSSION TO THE THESIS
GENERAL DISCUSSION

This thesis has examined several aspects of transduction by locust photoreceptors that have not been previously reported in papers that have investigated transduction by photoreceptors of other invertebrate species. Where a clear similarity has been shown to exist between a property of the locust photoreceptor and those of photoreceptors that have been more intensively investigated, I have tried to present a sketch of that property, rather than an attempt to repeat, in detail, previous experiments that have been performed on other preparations. The conductance mechanisms that generate the light-induced depolarization are an example. These appear to be similar to those that have been reported in studies on the photoreceptors of Limulus and barnacle (Millecchia and Mauro, 1969; Brown et al, 1970; Hanani and Shaw, 1976). The results of chapter one will not, therefore, be discussed any further. This discussion will concentrate on the results of chapters two, three and four and it will attempt to suggest further experiments on the pharmacology of transduction by locust photoreceptors.

Chapters two, three, and four show that superfusion of the retinular cells with salines that contain azide, \( N_2 \) (anoxic), fluoride, or neutral anaesthetics causes a noisy depolarization of the receptor. Further discussion of this phenomenon will be split into sections that will deal separately with the kinetics of the voltage noise produced by these chemical treatments and with the biochemistry that underlies their action.
The kinetics of the chemically-induced noise

Chapter three presents evidence that the chemically-induced voltage noise arises from fluctuations in the light-sensitive conductance. It is proposed in the discussion section of chapter three that spontaneous activity of an intermediate in the transduction mechanism is responsible for both the increased sodium conductance of the receptor and also for the voltage noise. Because the size of the chemically-induced voltage event is much smaller than the single photon event, it is suggested that the intermediate responsible for the noise occurs after the major amplification step in transduction. In the model of fig. 3.10 (reproduced overleaf) the active enzyme $E_2^*$, or the active transmitter $T^*$ are suggested as candidates for the intermediate that causes the chemically-induced noise. Azide and fluoride are presumed to block this intermediate's inactivation, while neutral anaesthetics activate the intermediate directly. We will now take a closer look at the model of fig. 3.10 and try to reconcile its predictions with the hypothetical model for the generation of transducer noise and light-induced noise that was presented in pages 5 to 8 of the general introduction to this thesis.

The voltage noise that is seen following the treatment of the photoreceptors with fluoride mimics the light-induced noise that can be recorded from the same photoreceptor when it is strongly illuminated and is therefore light-adapted. If $T$ is the intermediate that is responsible for the fluoride-induced noise, then, according to the scheme of fig. 3.10, fluoride would create a constant concentration of $T^*$ in darkness by blocking the inactivation of a fraction of the active transmitter molecules. Since the concentration of transmitter
A scheme for phototransduction whereby the effects of fluoride might be explained. The absorption of a photon by rhodopsin causes the conversion of rhodopsin to an active metarhodopsin, M-R* (Hamdorf & Kirschfeld, 1980). M-R* initiates an enzyme cascade after a delay time $\Delta t$. The cascade results in the production of a burst of active "internal transmitter", T*. It is suggested that fluoride blocks the inactivation of a later stage in transduction, for example the conversion of T* to its inactive form $T^i$, or $E_2^*$ to $E_2^i$. 
is now constant, the noise that is induced by fluoride must arise solely from the random nature of the interactions between active transmitter and ionic channels, so as to open the latter. This would seem to imply that, when the light intensity is sufficiently strong for light-induced noise to mimic fluoride-induced noise, the light-induced noise is likewise generated only by the interactions of transmitter and channels. Photon shot noise would not, therefore, appear to significantly contribute to light-induced noise at these light intensities. The latter conclusion of our argument seems, however, unlikely, as we shall see below.

A simple, approximate, calculation based on Campbell's theorem (see appendix, page 111) shows that, if each photon produces a puff of transmitter that lasts for 5 ms, then, at the light intensity used in fig. 3.2, at which light-induced noise is identical to fluoride-induced noise (3 x 10^{13} photons.cm^{-2}.s^{-1}), the relative magnitude of the fluctuations in the concentration of the internal transmitter caused by photon shot noise might be the same as the relative magnitude of the conductance fluctuations that can be inferred from the light-induced voltage noise. Thus it is likely that photon shot noise does significantly contribute to the light-induced noise at those light intensities at which light-induced noise is mimicked by fluoride-induced noise. If this is the case, then some source of fluctuation in the concentrations of E^* or T^* similar to that caused by photon shot noise must also exist in the fluoride-treated photoreceptor to account for the mimicry of light-induced noise by fluoride-induced noise.

One possibility is that fluoride causes E or T to cycle between active and inactive states, rather than causing a complete blockage of the inactivation process in some molecules of E^* or T^*.
The resulting fluctuations in $E^*_z$ or $T^*$ will mimic the fluctuations caused by photon shot noise. If such fluctuations do occur, then the 'shot event amplitude' of the chemically-induced noise that is calculated in chapters two, three, and four represents an upper limit to the true size of the chemically-induced event because of the extra variance contributed to the noise by the fluctuations in the active intermediate.

In view of the above considerations, a first step in the further analysis of the chemically-induced noise might be to determine the relative contributions of photon shot noise and transducer noise to the light-induced noise recorded at the light-intensities at which chemically-induced noise mimics light-induced noise. A careful estimate of the quantum efficiency of the photoreceptor, coupled with measurements of the sensitivity of the photoreceptor when strongly illuminated, may allow a comparison to be made between the expected photon shot noise contribution and the light-induced noise that is actually recorded (cf. Lillywhite and Laughlin, 1979).

Another area of the kinetics of the chemical effects that might be usefully investigated is a study of the afterpotentials that follow the response of cells to light flashes after they have been treated with fluoride or azide. These afterpotentials should be re-examined using weaker flashes of light, preferably within the linear range of the photoreceptor's response to light. Estimates could then be made of the time-course with which the afterpotential develops during the response and of the number of active intermediates released per photon. The model of fig 3.10 predicts that the time-course of the generation of spontaneously active
intermediates should follow the time-course of the receptor potential.

The above discussion of the kinetics of the chemically-induced noise has made it clear that several more experiments will have to be performed before a quantitative model, based on fig. 3.10 can be attempted. Fig. 3.10 provides only a qualitative, and perhaps naive, framework for the development of quantitative solutions.

The biochemistry of the chemically-induced noise

The results of chapters two, three and four may provide some biochemical markers (albeit crude ones) to use in screening enzymes extracted from insect photoreceptors for a possible role as an intermediate in the transduction process. The results suggest that one such enzyme will be activated by azide, anoxia, fluoride and neutral anaesthetics.

We can also examine the properties of enzymes that are currently thought to be regulators of biological activity in other tissues for evidence that they are activated in the presence of the chemical agents used in this thesis and therefore might be candidates for the proposed intermediate. In particular, the enzymes that catalyse the synthesis and degradation of cGMP and cAMP have received a lot of attention recently.

Several authors have suggested that cGMP is the internal transmitter of transduction in the outer segments of vertebrate
rod photoreceptors (review: Bownds, 1980). Rapid reductions in the intracellular concentration of cGMP can be observed on illuminating intact frog rod outer segments (Woodruff et al, 1977). cGMP is also known to activate a protein kinase that maintains two small proteins in the outer segment in a phosphorylated state. (Polans et al, 1978). Bownds (1980) suggests that these small proteins may regulate the permeability of the membrane of the outer segment. In the phosphorylated state, Bownds proposes that the small proteins maintain a high permeability of the plasma membrane to sodium ions. Light initiates a fall in the concentration of cGMP through the activation of a cGMP-ase in the disc membranes of the outer segment. The lowered concentration of cGMP results in a dephosphorylation of the small proteins through phosphatase activity and so a reduction in the sodium permeability of the photoreceptor's plasma membrane. A hyperpolarization of the photoreceptor then develops, as can be observed electrophysiologically (review: Fain and Lisman, 1981). By this scheme, cGMP is presumed to be an internal transmitter that increases the conductance of the rod photoreceptor's plasma membrane. If cGMP were presumed to be the transmitter for invertebrate photoreceptors, then an increase in the cGMP concentration might be expected upon illumination so as to produce the increased conductance that is typical of the illuminated membranes of invertebrate photoreceptors. Guanylate cyclase, the enzyme that catalyses the synthesis of cGMP might therefore be an intermediate in transduction by invertebrate photoreceptors.

Fein and Corson (1979) have suggested that an enzyme with similar pharmacological properties to adenylate cyclase (which catalyses the production of cAMP) may mediate a stage of
transduction in *Limulus* photoreceptors. They base their claim on their observation that fluoride, vanadate, and non-hydrolysable analogues of GTP cause spontaneous activation of the light-induced sodium conductance. All three of these agents are known to activate adenylate cyclase (Schramm and Ester, 1970; Perkins and Moore, 1971; Shinozawa et al, 1979).

However, no conclusive evidence for the involvement of cyclic nucleotides in phototransduction has yet been obtained for either vertebrate or invertebrate preparations.

As regards the agents used in this thesis, as noted above, fluoride activates adenylate cyclase *in vitro* (Schramm and Ester, 1970; Perkins and Moore, 1971). Fluoride is also able to activate guanylate cyclase obtained from some preparations (Goldberg and Haddox, 1977). Azide and anoxia are able to increase the levels of cGMP present in some tissues (Murad et al, 1978; Goldberg and Haddox, 1977). Triton X100, a lipid soluble detergent, is able to activate both adenylate and guanylate cyclases (Perkins and Moore, 1971; Goldberg and Haddox, 1977). The action of Triton X100 might have some analogies with that of the neutral anaesthetics used in chapter four of this thesis.

The presence of a transduction intermediate that is an enzyme with pharmacological properties similar to adenylate and guanylate cyclase would, therefore, be consistent with the effects of the chemical agents used in this thesis.

We must, however, be cautious of being too simplistic in our interpretation of the pharmacology, when we insist that all the
chemical agents used in this thesis directly affect a single enzyme. The recent work on the biochemistry of the control of cGMP concentrations in rod outer segments shows that there are many physiological agents and metabolites that modulate the activity of the system at several stages. ATP phosphorylates rhodopsin (review; Kuhn, 1981) and also mediates a reduction in the sensitivity to light of the light-activated cGMP-ase (Liebman and Pugh, 1978; Kawamura and Bownds, 1981). Internal calcium concentrations may also modulate the sensitivity of the light-activated cGMP-ase (Kawamura and Bownds, 1981). GTP is a necessary co-factor in the activation of the light-activated cGMP-ase and a GTP-ase step is involved in the inactivation of the cGMP-ase (review; Shinozawa et al, 1979). Lastly, the phosphorylation of small proteins by a cGMP dependent protein kinase may play a role in the modulation of the plasma membrane permeability (Bownds, 1980) so that ATP is a necessary co-factor in the action of cGMP on its target proteins.

Given such a multiplicity of controls by cellular metabolites in the light-dependent biochemistry of a putative internal transmitter, cGMP, we should not be surprised, perhaps, that the variety of chemical agents used in this thesis all act so as to increase the spontaneous activity in the phototransduction mechanism.
The ratio between the standard deviation of the transmitter fluctuations caused by photon shot noise and the mean transmitter concentration is given by Campbell's theorem (cf. Fain and Lisman, 1981).

\[
\frac{\text{s.d.}}{\text{mean}} = \sqrt{\frac{1}{n \cdot T}}
\]  

where \( n \) is the mean light intensity in effective photons per receptor per second and \( T \) is the duration of the puff of transmitter that each photon releases.

For the data of fig. 3.2, the light intensity used was \( 3 \times 10^{13} \text{ photons.cm}^{-2}.\text{s}^{-1} \) which corresponds to approximately \( 3 \times 10^4 \) effective photons per receptor per second (page 18). Therefore, for \( T = 5 \text{ms} \), Eq. 1 yields a value of 0.08 for the s.d.: mean ratio of the transmitter fluctuation: It must be stressed that the approximate nature of the conversion to effective photons per receptor per second makes this estimate accurate only to an order of magnitude.

The variance of the photon shot noise in the light-adapted state illustrated in fig. 3.2 was 0.6 mV\(^2\) after correction for non-linear summation. The ratio between this figure and the mean depolarization, 15 mV, yields a value of 0.001 \( G_0 \) for the variance : mean ratio of the light-induced conductance fluctuations (see Katz and Miledi, 1972), where \( G_0 \) is the resting conductance of the cell. The mean light-induced conductance at a depolarization of 15 mV is, assuming self-shunting, equal to 0.5 \( G_0 \). The ratio between the s.d. and the mean of the light-induced conductance is therefore equal to:

\[
\sqrt{\frac{0.001 G_0}{0.5 G_0}} = 0.045
\]

which is of the same order of magnitude as the s.d. : mean ratio of the fluctuation in transmitter concentration caused by photon shot noise.
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