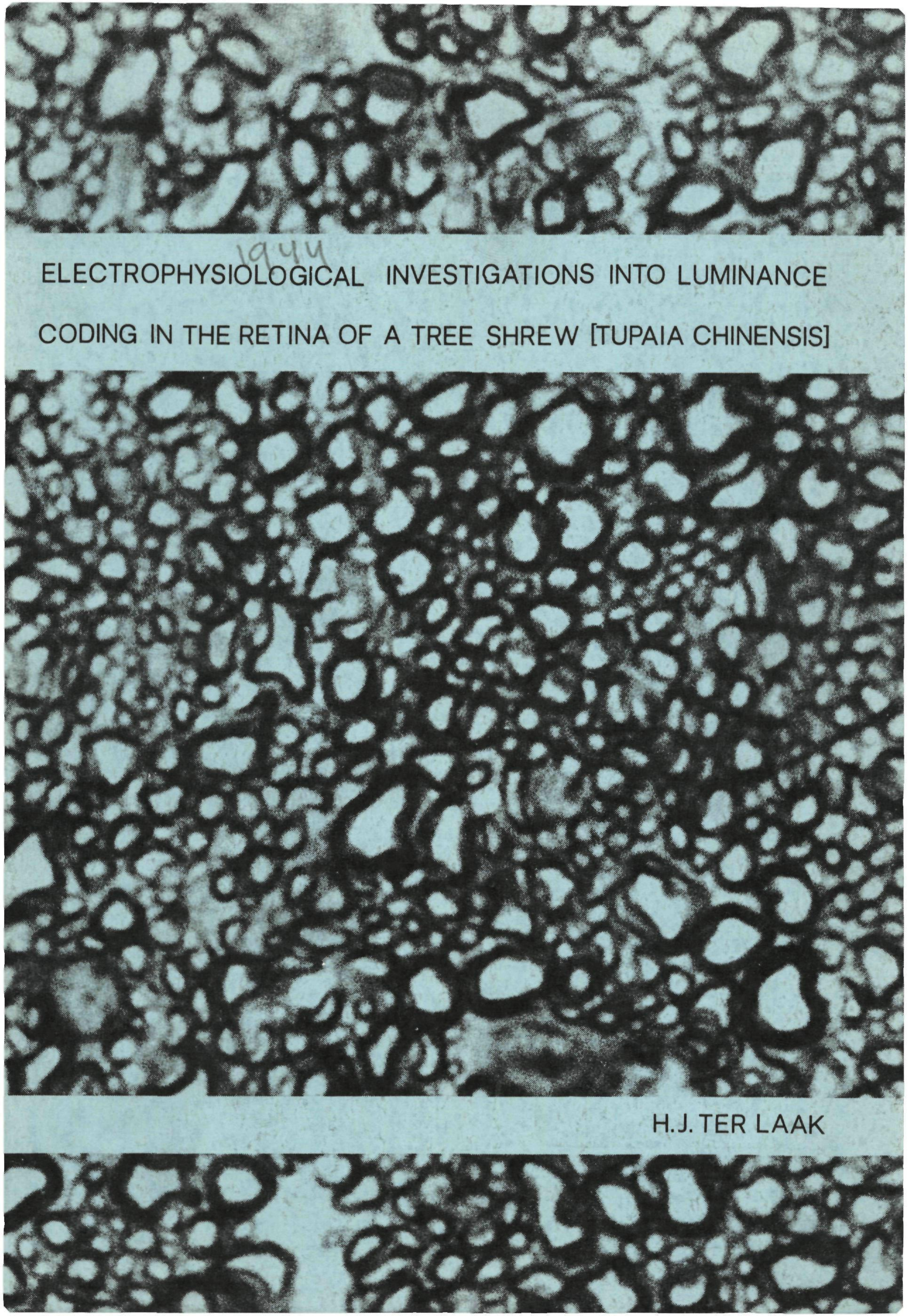

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ELECTROPHYSIOLOGICAL INVESTIGATIONS INTO LUMINANCE CODING IN THE RETINA OF A TREE SHREW [TUPAIA CHINENSIS]

H.J. TER LAAK

ELECTROPHYSIOLOGICAL INVESTIGATIONS INTO LUMINANCE CODING IN THE RETINA OF A TREE SHREW (TUPAIA CHINENSIS)

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ELECTROPHYSIOLOGICAL INVESTIGATIONS INTO LUMINANCE CODING IN THE RETINA OF A TREE SHREW (TUPAIA CHINENSIS)

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Aan mijn vader en moeder

Omslag: Licht-mikroskopische opname van een "semi-dun" dwarscoupe (1µ) van de Nervus Opticus (*Tupaia chinensis*) Totale vergroting: 4200 x.

CONTENTS

CHAPTER 3 TWO TYPES OF ON-CENTER UNITS IN THE ALL-CONE RETINA OF *TUPAIA CHINENSIS*

CHAPTER 4 INFLUENCE OF ADAPTING SPOTS ON THE RESPONSES OF ON-CENTER UNITS IN THE ALL-CONE RETINA OF TUPAIA CHINENSIS

CHAPTER 5 RESPONSES OF RETINAL ON-CENTER FIBRES IN *TUPAIA* AND HUMAN PSYCHOPHYSICS: COMPARISONS AND CONSIDERATIONS IN THE LIGHT OF THE THEORY OF SIGNAL DETECTION

GENERAL INTRODUCTION

The experiments described in this thesis were carried out on tree shrews; *Tupaia chinensis*. This animal inhabits the jungles of South East Asia. The genus *Tupaia* belongs to a group of primitive monkeys, viz. to the suborder *Prosimiae*.

In evolutionary terms *Tupaia* is considered to be a descendent of a species which was the common ancestor of the animals now belonging to the orders *Insectivora* and *Primates*. The high development of the visual system is one of the characteristics of *Primates*, just as is its arboreal mode of life. *Tupaia* is the only Primate with an 'all-cone' or, to express it somewhat more carefully, with a 'cone-dominated' retina. This is the most important reason why this animal was selected for electrophysiological studies on the visual system.

In psychophysical experiments in man or in behavioural experiments with other animals one can describe the properties of the visual system; what is then found is the input - output relationship of a whole system. What happens inside the structures belonging to the visual system is unknown. This black box can be broken open by trying to isolate responses from individual structures. This was achieved in the study reported here using two types of electrodes; macro- and micro-electrodes. With macro-electrodes responses from the receptors and the cells of the inner nuclear layer of the retina (electroretinogram; ERG) were obtained and with micro-electrodes responses from single optic tract fibres.

In this thesis the sequence of the chapters follows the steps leading to the desired correlation with psychophysics (chapter 5).

In chapter 1 a method is described with which it is possible to minimize the movements of the animals during measurements with micro-electrodes. The influence of anaesthesia on the visual parameters is also considered in this chapter.

The 'all-cone' nature of the retina of this animal is examined in chapter 2 with the aid of the results from experiments using macro-electrodes (ERG).

In chapter 3 responses of single optic tract fibres, measured with micro-electrodes, are reported. The fibres responding to the onset of light (on-center units) have been studied extensively. Two types of these units were found.

Properties of the two types of units in the dark adapted state, such as the center size and the location at the retina, have been studied. A superposition model for both types is presented from the application of which the responses can be interpreted. The significance of these studies for perception is discussed. The appendix to this chapter gives a survey of the several types of units found in Tupaia.

The characteristics of the on-center units in the light adapted state are considered in chapter 4. The model presented in chapter 3 has been further tested with experiments concerning the adaptation pool and with experiments in which centered adaptational spots were used.

In chapter 5 the results from the two types of units are considered in the light of the Theory of Signal Detection. Two models based on this theory are often applied in psychophysics, viz. the 'multirange meter' model and the 'multiplicative noise' model. The variability of the responses of the two types of on-center units appear to be more additive in the one and more multiplicative in the other. Thus these two types of units appear to coincide with the two models mentioned above.

CHAPTER 1

A METHOD FOR PROLONGED ELECTROPHYSIOLOGICAL EXPERIMENTS WITH A TREE SHREW (TUPAIA CHINENSIS)

1.1. Introduction

The tree shrew (Tupaia chinensis) has been selected for our electrophysiological study of the visual system for two reasons. According to current opinion, the retina of the animal contains a single kind of photoreceptor, i.e. cones (Samorajski et al., 1966). However, Dieterich (1969) presented some evidence that the rod-like cones may be real rods. Furthermore Tupaia is considered to be standing at the base of primate phylogenetic development (see for instance Hafleigh and Williams, 1966, Schwaier, 1973), which makes it easier to correlate certain aspects of the visual system of the animal with human visual perception.

Our electrophysiological studies using macro- or micro-electrode techniques can be carried out only with an animal completely immobilized. No adequate methods for anaesthetizing or paralyzing the animal for long periods in a stable condition have been described in the literature. Moreover, it is known that neural responses are very much dependent on the physiological state of an animal and on the depth of the anaesthesia. The intraperitonial administration of sodium pentobarbital as was used by Berger (1968) cannot, therefore, be used in our experiments.

Therefore the effects of the anaesthetic halothane on the electroretinogram (ERG), on the response of single optic tract fibres, on the heart rate and on the carbon dioxide content of the expiratory air have been studied. Because there are already visible effects with percentages that are too low to suppress movements sufficiently, paralysis of the animal is necessary during the experiments. The results of our study regarding optimal artificial ventilation under conditions without halothane will also be presented. The resulting procedure makes it possible to conduct experiments lasting for 12 to 18 hours.

Some technical problems such as the construction of an endotracheal tube, the measurement of the respiratory CO_2 content, and a method for sampling arterial blood will be discussed below.

1.2. Methods

1.2.1. Management of *Tupaia chinensis*

The animals were imported from Thailand and kept in quarantine for three weeks. They were placed in separate cages at a temperature of 24 °C. The cages measured 600 x 450 x 350 mm and an upside down flower-pot served as a hiding-place. The daily diet consisted of a quarter of an apple or an orange, 10 grams of meal worms and 25 grams of meat mixed with protein rich food (Pelsifood ^{R} ; Trouw & Comp., The Netherlands) containing 0.6 milligrams vitamin C. The animals were examined for the presence of intestinal *Strongyloides* and if necessary treated with tetramisole hydrochloride (Ripercol ^{R} ; Janssen Pharmaceutica, Belgium). It appears that the presence of these worms influences the body weight markedly. The average body weight of the animals, both males and females used in this study was 160 grams.

1.2.2. Construction of the endotracheal tube

The main part of this tube are three polyethylene tubes: one with an outer diameter of 2.0 mm and a wall thickness of 0.25 mm (A in Fig. 1.1) and the other two with outer diameters of 0.9 mm and wall thickness of 0.2 mm (B and C in Fig. 1.1). The length of tube A is 100 mm, that of tube B and C 120 mm.

Tube B used for sampling the expiratory air, is pushed through a small hole in the main tube A at 10 mm from the end and glued with Bisonkit (Perfecta Chemie, The Netherlands).

Tube C used for blowing up a cuff, is glued on the main tube A 15 mm from the end. Next, two rings of Bisonkit are made at 5 and 20 mm from the end of A and dried for 24 hours.

The cuff is made by plunging a glass rod (2.5 mm diameter) several times into a vulcanized latex solution in ammonia

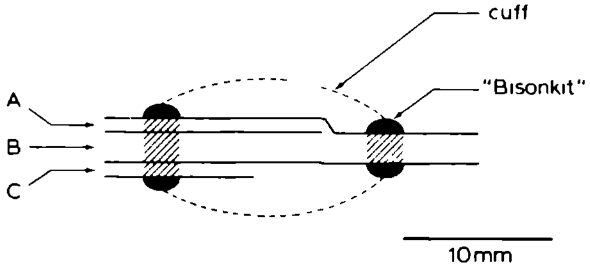

Figure 1.1 Schematic diagram of the end of the endotracheal tube.
A. Central tube for ventilation
B. Tube for sampling the expiratory air
C. Tube for blowing up the cuff

(Valjex, type L5, Van Roon & Comp., The Netherlands). After drying, the cuff is placed on the tube so that both of the rings are completely covered. Firm thread is wound around the ends of the cuff and glued with Bisonkit. After a further 24 hours drying period the now complete endotracheal tube is plunged in the latex solution once more.

1.2.3. Measurement of the carbon dioxide percentage in the expiratory phase

The sampling tube (B, Fig. 1.1) of the endotracheal tube is connected by 2 m. polyvinylchloride tube to the capnograph (Fig. 1.3). The analyzer of this instrument is connected with a flow inducer (Peripomp, Wilten & Comp., The Netherlands), that ensures a flow of 6 ml/min. through the analyser. An electromagnetic shutter blocks the sampling tube except for a 0.2 sec period during the expiratory phase. This shutter is triggered by the ventilator. The trigger consists of the photoresistor placed in front of the control light of the ventilator (light on during inspiration). The resistor is then connected to an electronic timing device.

1.2.4. Measurement of the arterial carbon dioxide pressure

Blood samples are taken from the ventral tail artery by the following procedure. The tail is anaesthetized locally with a marcain solution (0.5%). The skin is opened for 30 mm close to the middle of the tail and the connective tissue is cut with a knife to expose 20 mm of the artery. Then the circulation of the tail is blocked and a small hole is made in the artery. A polyethylene tube (0.6 diameter, wall thickness 0.16 mm) is pushed 1-2 cm into the artery in the direction of the heart. The circulation of the tail is re-established and the tube is flushed with heparin in saline (0.5 mg/ml) to prevent coagulation. One hour afterwards a sample of 1 ml blood is taken. The analysis of pH, p_aCO_2 and p_aO_2 were carried out at 38 °C. The instruments used were: pH-meter, Radiometer type 22, pCO_2 electrode, Radiometer type E.5036, and a pO_2 electrode, Radiometer type E.5046.

1.2.5. Procedure

The animals were provisionally anaesthetized in a plastic bag with a mixture of oxygen (30%), nitrous oxide (70%) and halothane. Thereafter an intramuscular injection of atropine (0.3 mg/kg) was given and the animal was placed in the stereotaxic apparatus. The head of the animal was fixed by pegs in the ear canals, and the mouth was covered by an anaesthesia cone (Fig. 1.2). Anaesthesia was continued with the aid of this cone by using a mixture of 30% oxygen and 70% nitrous oxide.

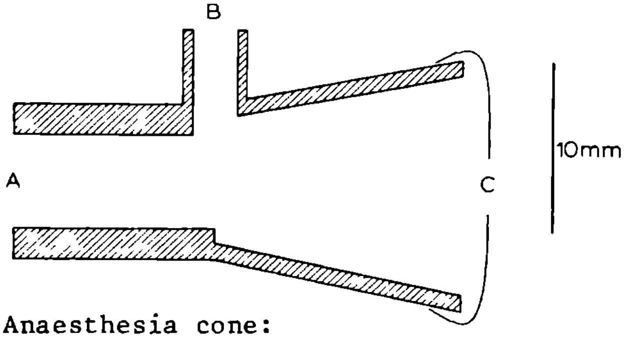

Figure 1.2 Anaesthesia cone:
A. Site of the attachment of the T-piece head of the Amsterdam Infant Ventilator
B. Expiratory air and excess inspiratory mixture outlet
C. Rubber membrane with a hole for the muzzle

The halothane was added to this mixture by means of a vaporizer. The electrocardiogram (ECG) of the animal was measured using subcutaneous electrodes in a fore and a hind limb. The ECG could be monitored on an oscilloscope and a heart rate counter, moreover the ECG was made audible via a loudspeaker. The body temperature was measured by a thermistor in the rectum and kept constant within 0.5 °C at 37.5 °C by an automatically controlled electrical heating mat. All pain causing contacts were locally anaesthetized with lidocaine (Xylocaine R ; Astra, Sweden) paste.

When the procedure outlined above was finished, the anaesthesia cone was removed and an endotracheal tube inserted into the trachea. This tube consists of a main tube for ventilation, a side tube for sampling the expiratory air and a cuff with a second side tube to block any leakage of gasses. Finally the animal was paralyzed by an intramuscular injection of gallamine triethiodide (Flaxedil; Specia, France) (14 mg/kg. hour) and artificially ventilated using the Amsterdam Infant Ventilator, AIV (Loosco, Amsterdam); see Fig. 1.3.

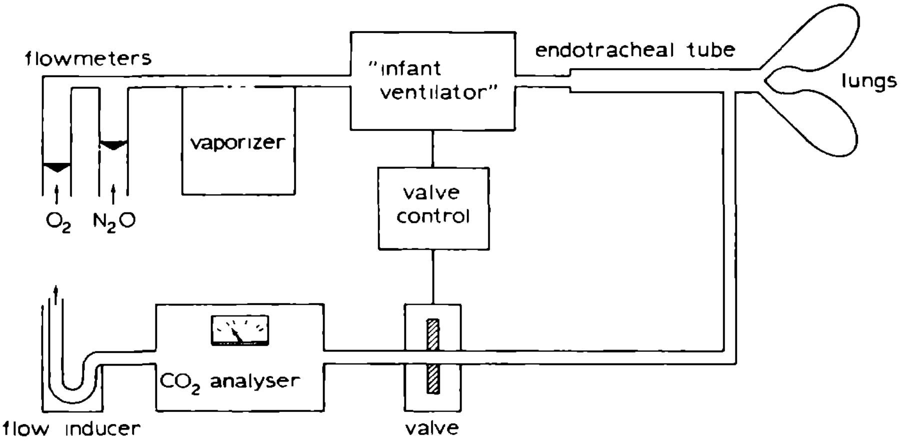

Figure 1.3 Diagram of the experimental equipment used for anaesthesia, artificial ventilation and CO_2 recording

The halothane vaporizer was disconnected when paralysis alone was necessary. It may be mentioned that the vaporizer causes a lowering of the inspiration pressure that cannot be neglected in quantitative measurements. The inspiration pressure is also influenced by small obstructions in the endotracheal tube. In this case the animal becomes hypoventilated, while the inspiration pressure is higher than normal.

1.3. Results

1.3.1. Correlation of the depth of the anaesthesia with electrophysiological and physiological parameters

The electroretinogram (ERG) and the responses of single optic tract fibres were used as an indicator of the influence of the level of anaesthesia. The ERG represents the macro response of the inner and the outer nuclear layer of the retina. The ERG was measured with a corneal contactlens containing a silver electrode. A reference electrode was placed under the tongue of the animal. The magnitude of the ERG is given by the peak to peak amplitude of the a-wave and the x-wave (see for instance Tigges et al., 1967).

The response of optic tract fibres was measured with microelectrodes (tungsten, tip diameter about 1 μm) and the average number of action potentials per second following a light flash was determined. Simultaneously with measurement of the ERG the heart rate was determined as an indication of the overall physiological condition of the animal.

The a- to x-wave amplitude of the ERG increases with increasing the percentage halothane from 2% to 4% and then decreases at still higher levels of anaesthesia (Fig. 1.4A). The latency of these two waves increases regularly (Fig. 1.4B). The heart rate decreases with increasing halothane from 3% to higher levels of anaesthesia (Fig. 1.4C). Hyperventilation induces an increase in the heart rate and in the ERG amplitude (Fig. 1.5 see x), whereas hypoventilation causes the opposite effects (Fig. 1.5 see y). The level of anaesthesia affects the optic tract fibre responses (Fig. 1.6) still more dramatically. The neural activity is doubled within a few minutes after the beginning of a 4% halothane anaesthesia and falls to about zero in 10 minutes with prolonged anaesthesia. The recovery after stopping the anaesthesia is rather fast

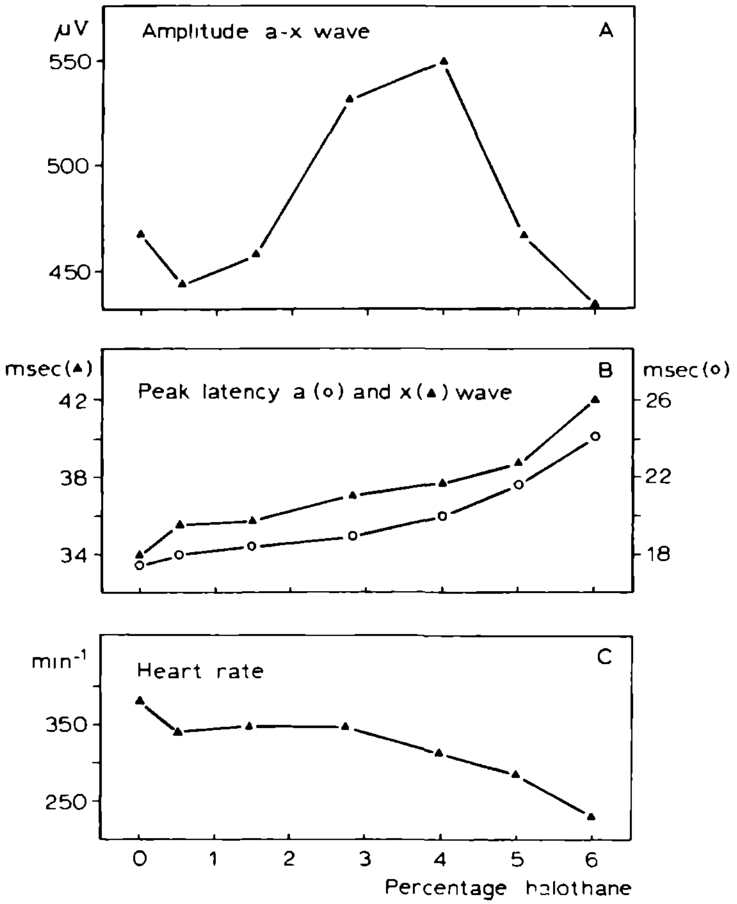

Figure 1.4 Influence of the anaesthesia on (A) the amplitude of the a-x wave of the ERG (B) the peak latencies of the a- and x-wave of the ERG and (C) the heart rate.
The results from 4 animals were averaged; periods with and without halothane alternated each other (two animals) and the anaesthesia was cumulative (two other animals). Measurements were carried out 5 - 10 minutes after the beginning of the anaesthesia.

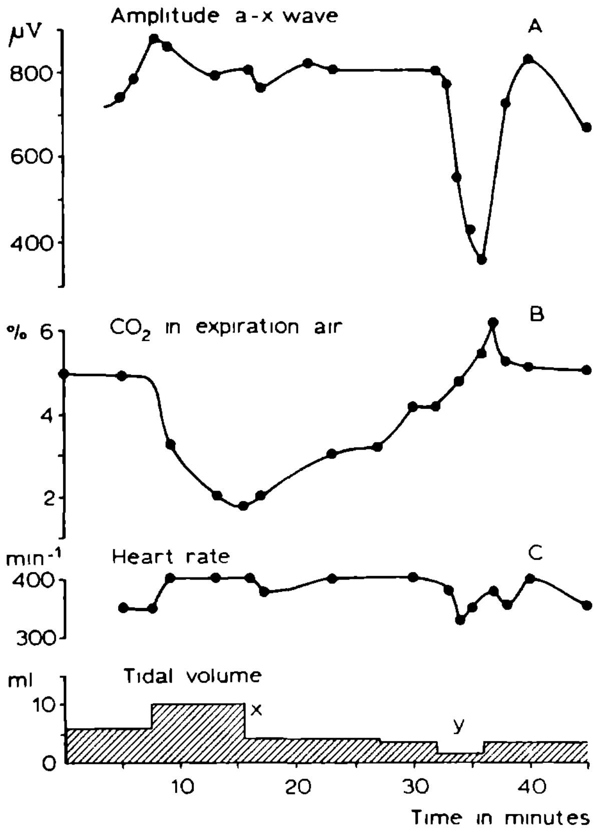

Figure 1.5 Influence of hyper (x) and hypoventilation (y) on (A) the ERG, (B) the percentage CO_2 in the expiratory air and (C) the heart rate. The tidal volumes are different at the beginning and at the end of this experiment, possibly because of an obstruction in the tube, which disappeared on changing these volumes.

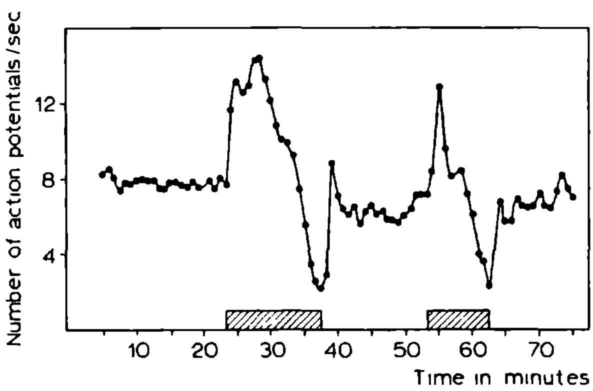

Figure 1.6 Influence of halothane (4%) on the firing frequency of a single optic tract fibre.

(within 2 minutes). The right part of Figure 1.6 shows that the halothane of the first anaesthesia period accumulates in the second period, because here the decrease to zero is much faster.

1.3.2. Optimal artificial ventilation

The CO_2 percentage of the expiratory air was measured continuously with a capnograph (Godard). For normal use of the capnograph a flow of 500 ml/min is needed. This quantity is too much for an animal as small as Tupaia. Therefore a device was developed for taking samples of only 6 ml/min in a part of the expiratory phase (Fig. 1.3). We now use a special type of capnograph, i.e. the infant capnograph (flow 60 ml/min) which makes our special device superfluous and which allows measurement of the CO_2 percentage during both the inspiratory and expiratory phases.

Additionally, samples of arterial blood were taken from the ventral tail artery to measure the partial pressure of carbon dioxide in the blood (p_aCO_2).

Optimal ventilation, to a first approximation, was determined under light anaesthesia without paralysis. The CO_2 percentage was measured when the artificial ventilation was just in phase with the spontaneous respiration. The animal was then paralyzed without using halothane while the mixture of O_2 and N_2O was kept constant. The CO_2 percentage was kept constant for one hour and the p_aCO_2 was then determined.

Although no value for the normal p_aCO_2 could be found in the literature, Altman and Dittmer (1964) give values of 35 to 40 mm Hg for most mammals. This range is confirmed by our data (Table 1.1). The p_aCO_2 values from the first two experiments are somewhat lower than those from subsequent ones, so hyperventilation cannot be excluded. These two experiments have been rejected and the remaining CO_2 values have been averaged. Averaging of all the results gives a CO_2 value of 5.0%. We now consider ventilation to be optimal when the CO_2 percentage falls in the range 4.5 to 5.5%. This holds true for a frequency of ventilation of 60 cycles per minute with a total flow of 450 ml per minute. The inspiratory phase lasts 0.3 - 0.5 sec (depending on the weight of the animal). The calculated tidal volume is then 2.25 - 3.75 ml (the 1 ml dead space in the ventilator included).

Under these conditions it is possible to maintain the paralyzed animals stable and in a good condition for 12 to 18 hours, and in most cases to keep the animal alive afterwards.

Animal no.	pH	p_aCO_2	p_aO_2	CO_2 percentage
F1	7.40	30 mm Hg	113 mm Hg	4.7
F7	7.25	31 mm Hg		5.4
	7.26	36 mm Hg		5.0
	7.22	41 mm Hg	87 mm Hg	5.3
F5	7.30	39 mm Hg	79 mm Hg	4.9
	7.31	37 mm Hg	84 mm Hg	4.2
K1	7.42	41 mm Hg	86 mm Hg	5.6
	7.45	34 mm Hg	130 mm Hg	4.7

Table 1.1. Results of bloodgas analyses and percentages CO_2 in the expiratory air.

1.4. Discussion

The body temperature and p_aCO_2 of Tupaia can be obtained from the data of other mammals (Altman and Dittmer, 1964). More recent data have been reported by Herbert and Mitchell (1971) who found for the cat a p_aCO_2 of 32.5 mm Hg, and by Domino et al. (1969) who gave a range of 38 to 42 mm Hg for the Primate Cynomolgus and the Rhesus monkey. The range of 34 to 41 mm Hg that is used by us for the p_aCO_2 of Tupaia therefore agrees with the physiological range in other animals.

Gerritsen (1971) found with ventilated rabbits that the b-wave amplitude of the ERG did not change using 1.3% halothane. During spontaneous breathing the same percentage caused a remarkable increase of the amplitude. In our experiments with ventilated tree shrews, the amplitude is also not increased by concentrations up to 1.5% (Fig. 1.4A). Hyperventilation causes a slight increase in the ERG amplitude (Fig. 1.5). This result is in accordance with those of Alpern et al. (1955) and Morgan and Ward (1970). The same effect is found using drugs causing vasodilatation (Henkes, 1951; Jacobs and Lincoln, 1954) and by increasing the oxygen supply in an isolated eye preparation (Niemeyer, 1974). It appeared to be impossible to suppress the reflexes and movements of Tupaia sufficiently with a halothane percentage lower than 1.5%. Therefore we decided to paralyse the animal and to maintain an artificial ventilation with a mixture of 30% oxygen and 70% nitrous oxide and to use local anaesthetics.

1.5. Summary

The effect of anaesthesia (halothane) on the physiological state of Tupaia chinensis has been studied by its effect on electrophysiological responses of the visual system (electroretinogram (ERG) and optic tract single fibre response) and on the heart rate. The animal was paralyzed and artificially ventilated with a mixture of 30% O_2, 70% N_2O and a small, variable, percentage of halothane.

The amplitude of the ERG was not influenced by halothane below a level of 1.5% but it increased with halothane percentages in the range 1.5% to 4%, and decreased at still higher percentages. The peak latencies of the ERG waves increased regularly. The firing frequency of single optic tract fibres

increased during the first 5 minutes of maintained 4% halothane anaesthesia, and decreased markedly thereafter reaching zero level at about 15 minutes. The heart rate decreased regularly with increasing halothane percentage, starting at 2.5%.

As the movements and reflexes were not sufficiently suppressed at halothane percentages below 1.5%, we preferred to paralyse the animal.

The optimal artificial ventilation without halothane was found by determining the p_aCO_2 and the CO_2 percentage in the expiratory air. The p_aCO_2 in our experiments varied from 34 to 41 mm Hg, which agrees with the values given for mammals in the literature. This range corresponded with a mean CO_2 percentage of 5.

Using this value we were able to conduct experiments with the *Tupaia* lasting 12 to 18 hours from which the animal subsequently recovered.

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CHAPTER 2

CHARACTERISTICS OF THE ERG OF TUPAIA CHINENSIS IN RELATION TO ITS CONE DOMINATED RETINA

2.1. Introduction

The receptors of Tupaia glis are believed to consist of cones only (Tigges, 1963; Castenholz, 1965), but there is some doubt concerning this all-cone nature. Samarajski et al. (1966) and Dieterich (1969) have shown that some rod-like photoreceptors (a few percent) are present. Polson (1968) showed with behavioural experiments that Tupaia is a dichromate, probably possessing a retina with 'blue' and 'red' cones only.

The investigations described in this chapter were carried out to answer the question whether or not functional rods are indeed present or not in Tupaia chinensis. In the experiments described in the following chapters, long during adaptation effects, caused by the rods if present, had to be carefully avoided. Because of this an animal with a cone dominated retina was chosen.

Tigges et al. (1963) have checked the ERG of Tupaia for characteristics typical for an all-cone retina. This was done by measuring stimulus response curves, latency curves and spectral sensitivity curves. In this chapter we present an extension of these studies on characteristics of the ERG for Tupaia.

Besides the absolute threshold and the latencies, the critical duration, the most sensitive frequency and the Critical Flicker Fusion frequency (CFF) in attenuation curves or so-called 'De Lange' curves and the time course of the dark adaptation were also measured.

It should be mentioned that this study is a preliminary study undertaken to investigate the presence of a homogeneous retina. The results presented in this chapter have therefore been focussed on this question.

The characteristics of the ERG of this animal will be compared with the characteristics of the ERG and with psychophysical data typical of rod or cone systems. The conclusion will be that all these data show that no rod function could be demonstrated in Tupaia chinensis.

2.2. Methods

The same methods as those described in chapter 1 were used. The animals were artificially ventilated with a mixture of O_2 and N_2O and were paralyzed with flaxedil (28 mg/kg/hour).

The ERG was recorded by means of a silver/silver chloride wire mounted in a contact lens. Both eyes were provided with the contact lenses, but the lens of the unstimulated eye was blackened. The contact lenses were self-fitting by means of the negative pressure caused by a water column (Fig. 2.1A). The ERG was measured differentially between the two contact lenses. The blackened one served as reference. With this type

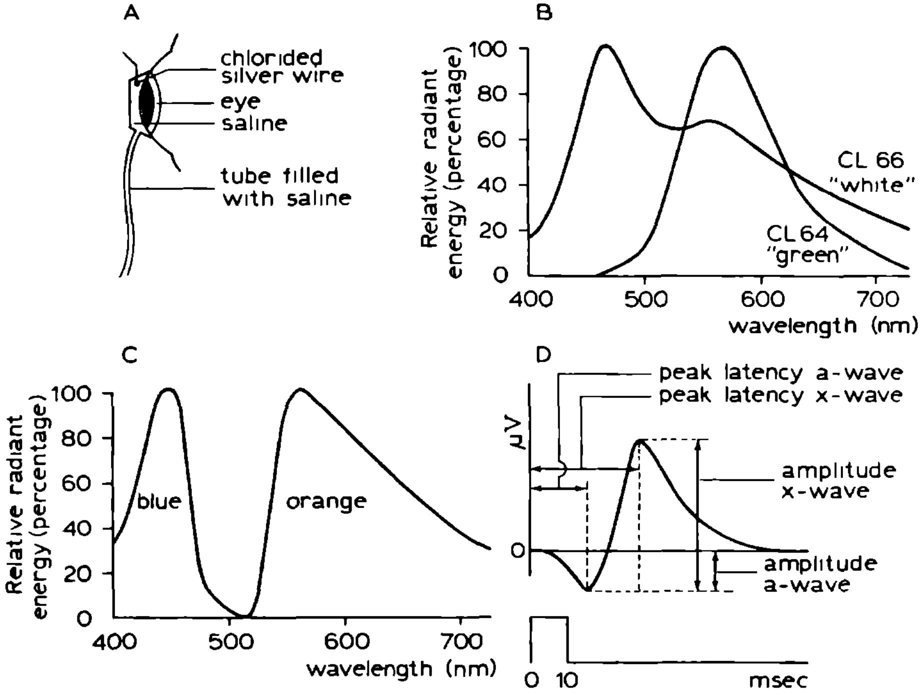

Figure 2.1. 1A: contact lens electrode; 1B: relative radiant energy of the green (CL 64) and the white (CL 66) cathode ray tube according to the manufacturer; 1C: relative radiant energy of the white cathode ray tube in combination with blue and orange filters (respectively BG3 and OG1; Schott-filters); 1D: data calculated from the electroretinogram (ERG) resulting from a 10 msec pulse.

of recording noise caused by the heart beat (ECG) was minimized. The animal was earthed subcutaneously. The ERG was amplified (0.2 - 250 Hz) and could be made visible on an oscilloscope. The ERG could also be averaged (Nuclear Chicago Data Retrieval Computer).

The stimulus was presented 20 - 50 times when rectangular pulses were used. When sinusoidally modulated light was used the number of presentations was dependent on the frequency used. With sinusoidal stimulation two lock-in amplifiers (Brookdeal) were often used. By means of this kind of amplifier the phase and the amplitude of the filtered response can be measured (Fricker, 1971; Padmos and Norren, 1971). In our study these two parameters were read from an oscilloscope screen as one dot. The data concerning the phase have not been presented because these data do not contribute to the problem of differentiating the rod from the cone function. The lock-in amplifiers filtered the original response in such a way that only the uneven harmonic components of the response were passed and the higher uneven harmonic components of the response were attenuated (with a factor n for the nth uneven harmonic). In some experiments a bandpass filter (Krohn-Hite; Q = 20) set to the stimulating frequency was used, so that mainly this component was passed.

The light sources consisted of cathode ray tubes: a green Ferranti tube, type CL 64 (Fig. 2.1B) and in some cases a white tube, type CL 66 (Fig. 2.1B) in combination with a blue or orange filter (Fig. 2.1C). These tubes were electronically controlled. The light from these sources could be sinusoidally modulated (0 - 99%) at different frequencies and mean levels. The light of the tube was concentrated on a diffuse screen placed 4 cm before the eye so that 80 degrees of the visual field was illuminated.

The amplitudes and the peak latencies were calculated as indicated in Fig. 2.1D. Wavelettes were often superimposed on the x-wave of the ERG. In these cases a mean curve was drawn through these wavelettes.

Light measurements were carried out with an AEG Luxmeter, type UM. The retinal illumination is expressed in human trolands (td). Because of the smaller size of the eye of Tupaia (about 3 x) with respect to the human eye, the calculated number of trolands for Tupaia was multiplied by a factor of 10, so that direct comparisons with human retinal illumination were possible.

2.3. Results

2.3.1. Stimulus response curves

Stimulus response curves were made in which the amplitude of the a- and x-wave was used as the response measure. When plotted on double logarithmic coordinates the lower part of these curves can be approximated by straight lines. With increasing intensities the response saturates and the curves level off.

In Fig. 2.2 stimulus response curves are presented for the a- and x-wave with the background illumination as the parameter (stimulus duration: 8 msec). It can be seen that

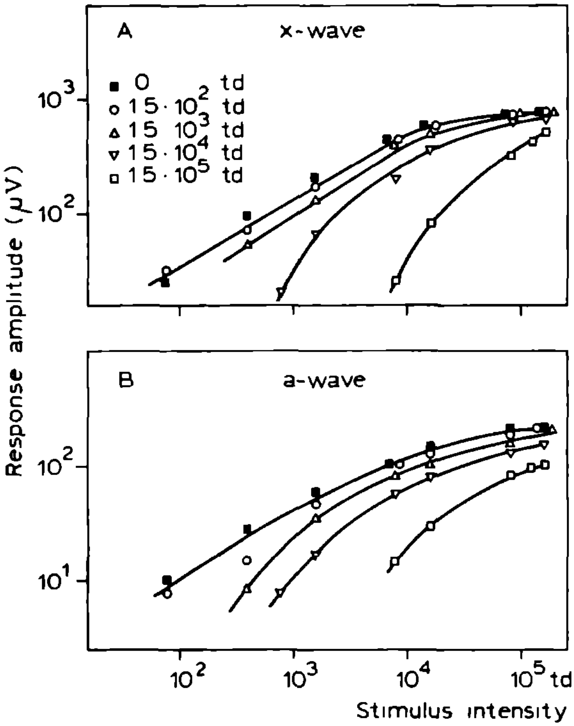

Figure 2.2. Stimulus response curves for the x-wave (A) and a-wave (B) amplitude with the level of background illumination as the parameter. Stimulus duration: 8 msec.

the slope of the lower part of these curves varies as a function of the background illumination. The slope-value increases with increasing background illumination and exceeds a value of 1 at high levels of background illumination. The responses saturate and the curves level off with increasing intensities. Furthermore, this figure shows that the dynamic range is shortened by high levels of background illumination (from about 2.5 log units in the dark up to 1.5 log units). The increase in the slope-values with background illumination was a general finding. Note that the stimulus response curves of the x-wave and the a-wave are apart from minor details, identical in shape and position with respect to the abscissa. In Fig. 2.3A an Increment threshold curve for the x-wave from two animals is plotted

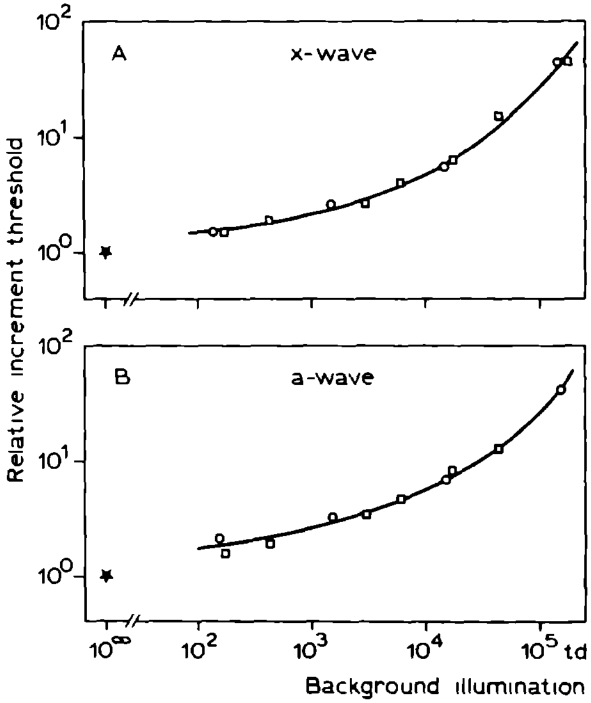

Figure 2.3. Increment threshold curves as a function of the background illumination for the x-wave (A) and the a-wave (B). The amplitude values at which the Increment threshold was measured were 100 µV and 25 µV for the x- and a-wave respectively. Stimulus duration: 5 msec (squares) and 8 msec (circles). The stimulus intensity in the dark adapted state was set to one (star).

as a function of the background illumination. In this figure the intensity referring to a fixed value of the response measure (amplitude x-wave) is plotted against different levels of background illuminations. It can be seen from this figure that the curves starts as an almost horizontal line. The slope of this line increases up to a value of 1 with the level of background illumination; in the last mentioned region a proportionality exists between the increase in the intensity and the background illumination and here the curve obeys Weber's law. It will be clear that other values for the response measure will influence the shape of this curve, since the slopes of the different stimulus response curves (Fig. 2.2) are different.

The stimulus response curves of the amplitudes of the a- and x-wave yielded similarly shaped curves. So the shapes of the Increment threshold curves of the a-wave and of the x-wave are also similar (Fig. 2.3A and B). In Fig. 2.4 the amplitudes of the a- and x-wave of the ERG-recordings have

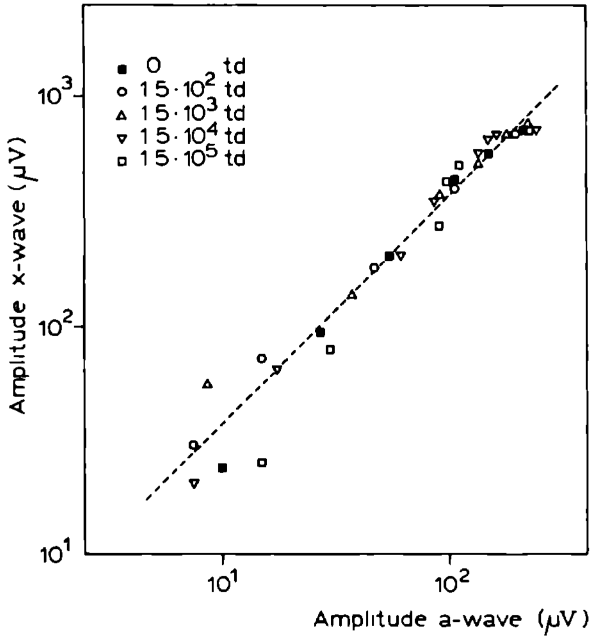

Figure 2.4. Amplitude values for the a- and x-wave at different levels of background illumination (indicated). The broken line indicates a proportionality. Stimulus duration: 10 msec.

been plotted on double logarithmic coordinates. It can be seen that the slope is about unity, indicating a proportionality between these two amplitude measures. It can also be seen that both this slope and the ratio between the amplitudes of the x- and a-wave are independent of the level of background illumination. In Table 2.1 the slope of these curves and the ratios of the amplitudes of x- and a-wave have been presented for four animals.

Animal no.	Slope	R
C3	1.00	3.7
F6	1.10	4.7
F7A	1.06	3.6
F7B	1.04	4.6
H6	1.30	4.8

Table 2.1. Slope values of x- and a-wave amplitudes on double logarithmic coordinates. R: ratio of x- and a-wave amplitude. The animal no. F7A and F7B concern two separate experiments with the same animal.

2.3.2. Peak latencies

The peak latencies of the a- and x-wave as a function of the stimulus intensity with the background illumination as variable are presented in Fig. 2.5 (in this figure the peak

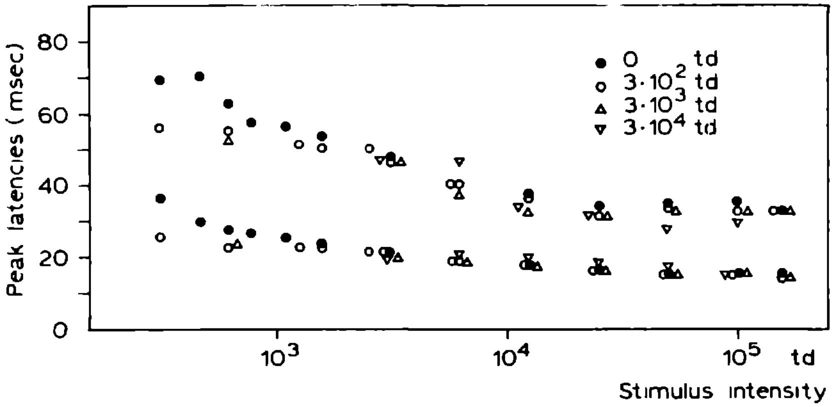

Figure 2.5. Peak latencies of the x-wave (upper part) and the a-wave (lower part) at different levels of background illumination (indicated). Stimulus duration: 10 msec.

latencies of the x- and a-wave have been plotted in the upper and lower part respectively). The dependency of the latencies of the x- and a-wave on the level of background illumination is in general weak with respect to the dependency on the stimulus intensity. However, at low stimulus intensities there is a relatively great influence of the level of background illumination. The latencies decrease for both waves with increasing stimulus intensity up to an intensity level of 10^4 td; stronger stimulation does not change the latencies very much. The latency-values of the x-wave vary from about 70 msec to 30 msec and those of the a-wave from 30 to 13 msec. These values are of the same order as the values measured by Tigges et al. (1967) in the dark adapted state.

2.3.3. Critical durations

Complete temporal summation is present as long as $I \cdot t = c$ for a given response measure (Bloch's law). Measurements of the upper limit of t for which this law holds true, the critical duration, are described in this section for different background illuminations. In general the amplitude of the x-wave was used as the response measure Fig. 2.6A and B show plots in which the amplitude of the x-wave has been plotted against the stimulus duration. Several stimulus durations were used while keeping the total energy in the stimulus constant. In most curves a horizontal part indicating the region of complete temporal summation can be seen. However, in some measurements a continuously declining curve was already observed from 1 msec on. If the later part of the stimulus does not contribute to the response then the resulting response will be inversely proportional to the stimulus duration. On double logarithmic coordinates a straight line with a slope of -1 will result. It can be seen from Fig. 2.6 that this is the case for most curves; so one can define in most measurements a measure for the critical duration, viz. the intersection of the horizontal line and the line with slope -1. Fig. 2.7 shows these critical durations as a function of the level of background illumination. It can be seen that the critical duration decreases and becomes approximately constant at the highest adaptation levels.

Although there is some scatter in Fig. 2.7 it can be seen that the critical duration is on the average 22 msec in

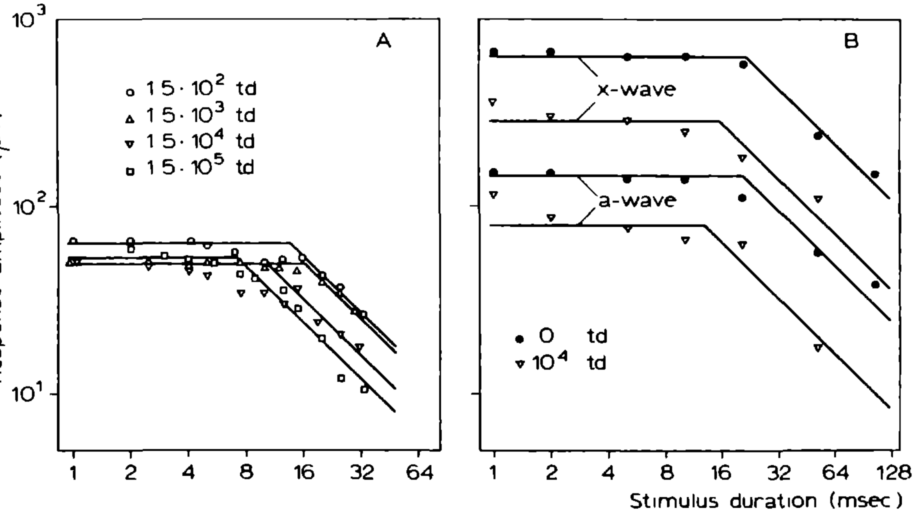

Figure 2.6. Amplitude values of the responses elicited by flashes (equal energy) as a function of the stimulus duration. A: x-wave amplitudes; B: x- and a-wave amplitudes. The levels of background illumination have been indicated. The horizontal parts of the curves obey 'Bloch's law'.

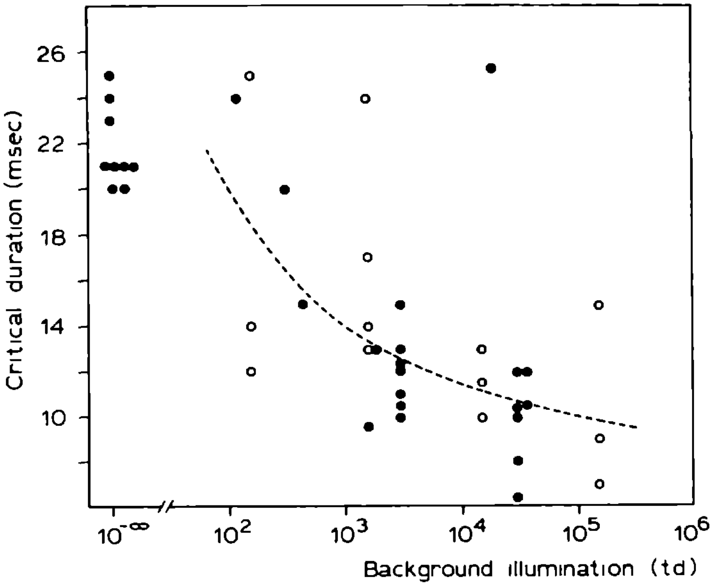

Figure 2.7. Critical durations as a function of the level of background illumination. Open circles correspond to low (< 100 µV) and closed circles to high (> 100 µV) response measures.

the dark adapted state and diminishes to 10 msec at the highest levels of background illumination.

The critical time for the a-wave equals the critical time for the x-wave (Fig. 2.6B) to a first approximation.

2.3.4. Attenuation curves ('De Lange' curves)

In each of the parts of Fig. 2.8 ERGs of about equal amplitudines have been placed together. It can be seen that the response shapes in the two figures are very similar. The response shape, however, changes as a function of the amplitude of this wave. It can also be seen that the response at a high level of background illumination is the shorter one in each figure and contains more high frequency components than the response at low levels of background illumination.

When a sinusoidally modulated background illumination is used and the resulting response is filtered by the lock-in amplifiers so that the response consists mainly of the first

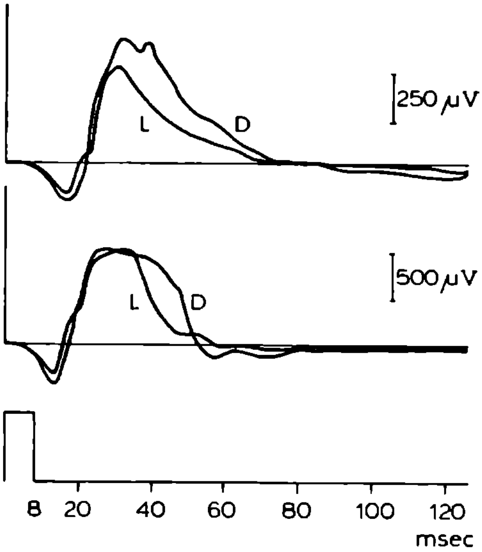

Figure 2.8. Superimposed ERGs. Responses of about equal amplitude have been placed together. D: dark adapted state; L: light adapted state (background illumination: 15,000 td).

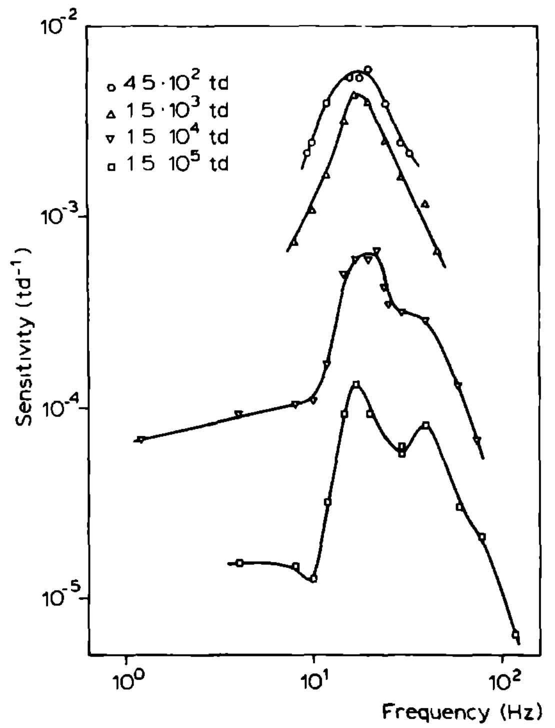

Figure 2.9. 'De Lange' curves with the background illumination as parameter (indicated). The constant criterium (output) method was used. The first harmonic components (measured with lock-in amplifiers) and in a lesser (1/3) degree, the third harmonic component are measured in the response (14 µV top-top). The point farthest to the right of each curve represents the CFC. Sensitivity has been expressed in td^{-1}, for which measure the intensity (td; half top-top values of the sinus wave) has been used.

harmonic components, 'De Lange' curves can be constructed (Fig. 2.9). On the ordinate the sensitivity is expressed in log td^{-1} and on the abscissa the log stimulus frequency has been plotted. Each point in this figure comes from a filtered response with a top-top value of 14 µV. It can be seen that different curves result from different mean background levels and that the sensitivity decreases as a function of the mean level of background illumination as in the Increment threshold curves (Fig. 2.3). All curves show a maximum sensitivity at about 16 - 18 Hz, independent of the mean level of background illumination. At higher levels of background illumination a shoulder and even a second peak at 35 - 40 Hz is observed, which is an expression of the high frequency content of the responses at high levels of background illumination. In Fig. 2.8 an increase in the high frequencies can be observed at high levels of background illumination. From Fig. 2.9 it can also be seen that the Critical Flicker Fusion frequencies (CFF) increase with increasing background illuminations. The CFF is defined here as the maximum frequency when the response criterium is reached with a 100% modulated stimulus (14 μV top-top values). At the highest background illumination a value of 120 Hz was found, which is in agreement with the data of Ordy and Samorajski (1968), who worked with unfiltered responses.

When the CFF-frequencies are plotted against log background illumination an almost straight line results (Fig. 2.10). The slope of this curve expressed in Hz/log unit background illumination, yields a value of 28.

Another type of 'De Lange' curve was measured by using a constant depth of sinusoidal modulation. Three peaks were found: at 4 Hz, 15 Hz and 40 Hz. The peaks at 15 and 40 Hz were also visible on using the constant criterium method at high mean background illumination. The 4 Hz peak was probably

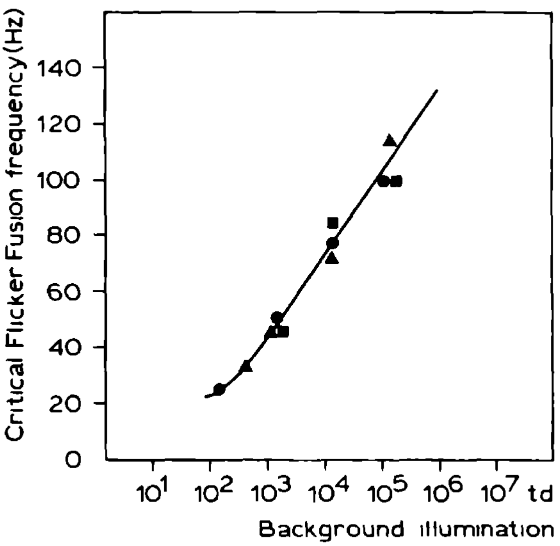

Figure 2.10. Critical Flicker Fusion (CFF) frequency as a function of the level of background illumination for 3 different animals.

caused by the second harmonic components. This peak was present in experiments in which blue, green and orange stimuli were used.

According to some authors the blue cone system is a slower system than the other cone systems (Norren and Padmos, 1973). The 4 Hz peak in our experiments was probably not caused by the blue system, but occurred as a result of the high degree of second harmonic components in the responses at low frequencies. However, some caution should be exercised because by using the lock-in amplifiers the 4 Hz peak in one experiment was exclusively found with a blue stimulus and an orange adaptation light, where by the red sensitive cones become much more adapted than the blue ones.

2.3.5. Dark adaptation curves

The time course of dark adaptation was measured in Tupaia by first exposing the eye to a strong stimulus (about 10^6 td) for 10 minutes and, after this strong light adaptation, measuring the x-wave amplitude (10 msec pulses) as a function of time. The constant output criterium method was used (so the stimulus intensity was decreased when the x-wave increased as a function of time). As the criterium a response-amplitude of 60 µV was chosen. The stimulus intensity required for this response decreased as a function of time, so the sensitivity of the cone system increased. First a rapid increase in the sensitivity occurred and subsequently the curve became horizontal after about 20 minutes (Fig. 2.11). In one experiment the sensitivity was measured over 90 minutes following the offset of light adaptation, but no further decrease in the curve was observed.

The same experiment was done with human subjects using the same stimulus apparatus. The pupils were dilated by means of atropine and the observers used the constant criterium method ('just visible'). Conventional dark adaptation curves were obtained, each showing a break at about 8 - 10 minutes after the beginning of dark adaptation (Fig. 2.11). The upper parts of these curves show the dark adaptation of the cone system, while the other parts of these curves show the adaptation of the rods, which is quite rapid after 8 - 10 minutes.

Such a break is not present in the dark adaptation curves of Tupaia.

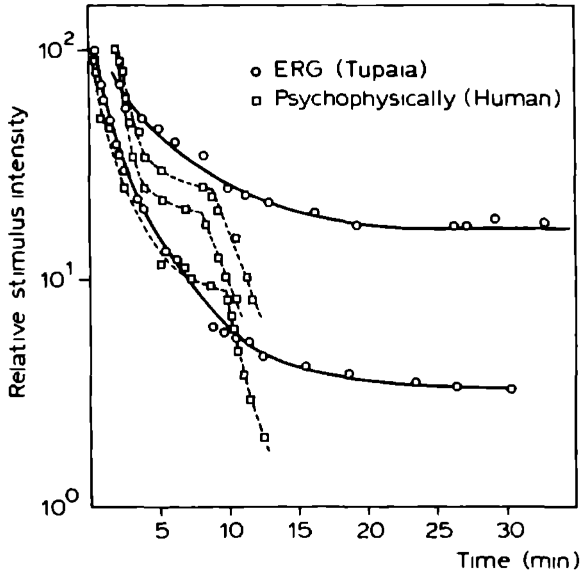

Figure 2.11. Dark adaptation curves for Tupaia (ERG; criterium: 60 µV) and man (psychophysically; criterium: just visible). The curves do not superimpose because of different experimental conditions (light adaptation).

2.4. Discussion

The shape of the stimulus response curves plotted on double logarithmic coordinates is in agreement with the results of Tigges et al. (1967). The lower parts of the curves can be approximated by straight lines. The slope of these lines increases with light adaptation. In the dark adapted state we found a mean slope-value of 0.7. This is also the case for the stimulus response curves presented by Tigges et al. (1967). If their curves are replotted, a slope of 0.7 in the dark and a slope of 1.0 in a light adapted state are found.

If stimulus response curves are replotted for the rat (Cone, 1963; Dodt and Echte, 1961) straight lines are also found. The slope of these lines is also less than unity in the dark: 0.83 for 20 msec pulses and 0.66 for 40 msec pulses.

Using long duration pulses (200 msec) Dodt (1962) found that the sensitivity of an all-cone animal (Sciurus sciurus) was about 5 log units lower than the sensitivity of the rat. Using short pulses we found that *Tupaia* was about 3 log units (measured in stimulus energy) less sensitive than the rat (Dodt and Echte, 1961).

The a-wave is absent from ERGs measured in the dark adapted state in both the rat (Cone, 1963) and man (Johnson and Bartlett, 1956). When increasing stimulus intensities are used the a-wave appears in the rat at an intensity which is about 3 log units higher than the intensity needed for the b-wave, and thus at the same level at which the stimulus response curves of *Tupaia* start. So it may be concluded that the a-wave is mainly generated by the cone system.

The shape of the Increment threshold curves of the ERG (Fig. 2.3) resemble those presented in chapter 4 (output of ganglion cells). This means that already on the level of the inner nuclear layer light adaptation influences the response amplitude. This has previously been reported for the ERG of man by Biersdorf (1965).

A remarkable finding was the fact that the a-waves in *Tupaia* were in most experiments roughly proportional to the x-waves; this is an indication that in *Tupaia* one system is operating, viz. the cone system.

Our latency curves from *Tupaia* resemble those of Tigges et al. (1967).

When we compare our peak latencies in the dark adapted state with those of the rat or man in the dark adapted state differences are again found. The peak latencies in the dark range from 70 - 30 msec for the x-wave and from 35 - 13 for the a-wave with increasing stimulus intensities for *Tupaia*. There is furthermore, only a slight dependence on the background illumination (Fig. 2.5). In man the peak latencies of the b-wave yield values of 125 - 150 msec in the dark and about 95 msec in a light adapted state. No a-wave was observed in these responses (Johnson and Bartlett, 1956). In the rat, in the dark adapted state, the peak latencies range from 140 - 60 msec for the b-wave and from 60 - 20 msec for the a-wave (if present; Cone, 1963).

The conclusion is that in the dark adapted state the latency of the x-wave in *Tupaia* is shorter than the peak latency in the dark adapted state of the b-wave in the rat and man. So *Tupaia* does not display the long latencies present in rod dominated or mixed retinas.

In the dark adapted state a critical duration of 22 msec was found for *Tupaia*. Cone (1963) found a value of 30 msec for the rat. If Cone used the true critical duration the

30 msec is possibly somewhat too short. Johnson and Bartlett (1956) found with the aid of the ERG critical durations of 100 msec for man in the dark and critical durations of 30 msec in a light adapted state when a stimulus of 7.5^o covering the fovea was used. The values measured by Troelstra (1964) for man (ERG) show the same dependency on the level of background illumination (120 - 20 msec). In psychophysics Roufs (1972a) found values ranging from 100 msec in the dark to 22 msec in the light adapted state using foveal (1^o) stimulation. In the peripheral retina Barlow (1958) found values ranging from 100 msec to 46 msec from the dark to the light adapted state with large field stimulation. Sperling and Jolliffe (1965) found shorter critical durations in the fovea than in the peripheral retina in the dark adapted state. Owen (1972) who found that Bloch's law was only valid within the spatial summation area, measured true critical durations of 90 msec in the dark and 52 msec in a light adapted state in the peripheral retina. It can be concluded from these data that cone vision and light adaptation yield shorter critical durations. Our values of 22 - 10 msec are similar to those measured in other psychophysical experiments at high levels of background illumination when foveal stimulation was used (Roufs, 1972a).

The 'De Lange' curves of Tupaia measured by means of the ERG (Fig. 2.9) show a maximum sensitivity at 15 - 20 Hz independent of the mean level of background illumination. The Critical Flicker Fusion frequency (CFF), however, is dependent on the background illumination.

On certain theoretical grounds (Roufs, 1972b) it would be expected that the maximum of the curve would be shifted to higher frequencies at high levels of background illumination. The data regarding critical duration and CFF are in agreement with the above cited theory (Roufs, 1972b). This discrepancy is explicable since the 'De Lange' curves display two maxima at high levels of background illumination; the maximum could be expected to be situated between these two.

Reuter (1972), working with rodent species, found that the maximum frequency sensitivity was dependent on the type of retina and the same dependence was found for the CFF. For the rat (rod dominated retina) the maximum sensitivity was found to be at a value smaller than 2 Hz, while the CFF was about 9 Hz. For the European squirrel (Sciurus vulgaris) with a cone dominated retina, values of respectively 12 and 45 Hz were found. Gouras and Gunkel (1962) found the maximum sensitivity at 2 - 4 Hz for the rat and at 9 - 10 Hz for the gray squirrel (Sciurus leucotis carolinensis); the latter values were independent of the level of background illumination as was also found for Tupaia (section 2.3.4). The ERG-CFF for some Primates were measured by Ordy and Samorajski (1968). For nocturnal (rod dominated) Primates (Aotus trivigatas and Galago crassicaudatus) the CFF was found te be 10 - 30 Hz depending on the level of light adaptation, while they found the CFF for Tupaia to be 90 - 100 Hz.

In human psychophysics the maximum sensitivity ranges from 3 - 12 Hz and the CFF from 7 - 50 Hz depending on the level of background illumination (Roufs, 1972a). Behavioural experiments with animals with cone dominated retinas also yield high maximum sensitivity values (Graf, 1973): the turtle (Chrysemys picta picta) at 8 - 9 Hz and the pigeon at 20 Hz.

From the above cited literature it will be clear that the most sensitive frequency-values and the CFF-values are higher, the higher the level of background illumination in mixed retinas; this is to be expected since cone function increases at high levels of background illumination. The frequency-values and CFF-values in other cone dominated animals such as Tupaia are in the same range as measured for Tupaia in this study.

The slope values for the straight line in the CFF-background illumination plot (Fig. 2.10) was found to be 28 (Hz/log unit background illumination). This value is close to the value found for the human eye (ERG), stimulated with rectangular pulses (light-dark ratio: 1), viz. 25 (Heck, 1957). For the pigeon-ERG a value of 43 was found (Hrachinova and Schmidt, 1968).

Attention must be given to the fact that with the constant input method in Tupaia more than one maximum was often found. It was not possible to isolate the maxima by using different coloured stimuli. The maximum at the highest frequency was found with intense levels of background illumination. The maximum at the lowest frequency (4 Hz) was found with green, blue and orange stimuli with 50% modulations. Norren and Padmos (1973) found that the shape of the ERG of the Rhesus monkey with blue and red stimuli was the same at low levels of background illumination. This is in agreement with the results of Tigges et al. (1967) who found identically shaped ERGs for Tupaia with different coloured stimuli in the dark and is confirmed by our own experiments (not presented here). It has already been mentioned that the second harmonic components play an important role at low frequencies, so that the 4 Hz peak is an effect caused by these components rather than an effect from the colour used.

In the curves resulting from stimulation with blue, orange or green a 4 Hz-peak is present. According to the data of Norren and Padmos (1973) it can be expected that the 'De Lange' curves for orange and green would show a peak at a higher frequency than 4 Hz. This was not observed in our experiments, which is probably due to the low levels of background illuminations used when comparisons of orange or green stimulation with blue stimulation were carried out.

The results obtained from dark adaptation curves indicate an homogeneous retina. Because of this it is improbable that the 4 Hz-peak found in the 'De Lange' curves should be ascribed to rods. Furthermore a high level of background illumination was often used for constructing the 'De Lange' curves measured with green stimuli, so the rods, if present, were saturated.

The dark adaptation curve of Tupaia does not show a break such as is found in the curves of human subjects (psychophysical experiments); this indicates that cones are dominating. The lack of such a break is no proof that the retina containes no rods. If a few percent of rods are present it is possible that the response measure (60 μV) will not be reached even with a dark adapted retina. The curve was roughly horizontal after about 20 minutes, while the curves of the human subjects declined markedly after about 10 minutes. The time constant of the logarithmic dark adaptation curves (Tupaia) was 6 minutes. However, by using a 15 Hz flickering stimulus a time constant of 3 minutes has been found. The latter result may tentatively be explained by the relative suppression of rod responses at higher stimulus frequencies. The dark adaptation curves of the rat (rod dominated) still decline several hours after light adaptation (Dodt and Echte, 1961). An almost identical curve to that found in Tupaia was obtained by Dodt (1962) by means of the ERG with Citellus citellus, a squirrel with an all-cone retina. About the same curve (behavioural experiments; time constant; 3 minutes) was found by Jacobs and Yolton (1971) with the ground squirrel (Citellus tridecemlineatus), an animal with an all-cone retina. However, Dodt (1962) obtained a curve (ERG) with a break from the 'all-cone' retina (as judged from light microscopy) of the European squirrel (Sciurus vulgaris) with blue or red stimuli, which was horizontal at about 30 minutes.

By means of behavioural experiments Maxwell and Granda (1972) concluded that in the retina of the turtle (Pseudemys scripta elegans) at least two receptor systems are present: one sensitive for long and one sensitive for short wavelengths. Stimulating with long wavelengths yielded dark adaptation curves in which the horizontal part was reached after 1.5 minutes and with short wavelengths after 45 minutes.

For mammals it is known that the rods are maximally sensitive at 500 nm. Energy of this wavelength was present in our stimulus. Our curves, however, do not show a break, so we conclude that our dark adaptation curves are mainly the results of the cone system.

2.5. Summary

1. ERG-recordings from Tupaia chinensis were carried out in order to investigate this cone dominated retina. The data are compared with data found in literature concerning rod dominated and mixed retinas.
2. The retina of Tupaia is about 3 log units less sensitive, as revealed by the ERG responses, than the rod dominated retina of the rat. The peak latencies of the a- and x-wave of Tupaia are shorter (25 and 50 meec).
3. The ratio of the x-wave and a-wave amplitude is about constant (on the average 4.3) under both dark and light adapted conditions.
4. The critical duration diminishes from the dark adapted state (22 msec) with increasing background illumination (to 10 msec).
5. The 'De Lange' curves show maximum frequency sensitivity at 15 - 20 Hz independent of the level of light adaptation and a Critical Flicker Fusion frequency (CFF) ranging from 35 up to 120 Hz dependent on the level of light adaptation. Other peaks were measured at 4 Hz and 40 Hz. The 40 Hz-peak was found at high levels of light adaptation. The 4 Hz-peak could not be ascribed with certainty to any particular cone type or to rods.
6. The time course of dark adaptation after exposing the eye to strong bleaching stimuli showed no break as seen in dark adaptation curves from mixed retinas. The time constant is 6 minutes as measured with short pulses (10 msec) and 3 minutes as measured with sinusoidal stimulation (15 Hz).

7. The results are compared in the Discussion with data from the literature concerning rod dominated or mixed retinas. The conclusion is that the ERG of Tupaia does not reveal characteristics present in rod systems.

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CHAPTER 3

TWO TYPES OF ON-CENTER UNITS IN THE ALL-CONE RETINA OF TUPAIA CHINENSIS

3.1. Introduction

In our investigation in which the results of measurements on single optic tract fibres have been correlated with the results from human psychophysics (chapter 5) two types of on-center units have been found. In our study only on-center units have been considered and short, 10 msec, pulses were applied. Long square wave pulses were also used for the characterization of the units. With these short and long square wave pulses it is possible to classify the on-center units into two types. A survey of all types found in our investigation will be presented in the appendix.

In the cat many authors have been able to divide retinal on-center units or off-center units into two types (Cleland et al., 1973; Ikeda and Wright, 1972; Enroth-Cugell and Robson, 1966; Winters et al., 1972; Fukuda, 1971); the information concerning other mammals is quite sparse. Gouras (1968) has reported the existence of tonic and phasic units for the Rhesus monkey which are correlated with colour sensitivity. Marrocco (1972) confirmed this finding but not the strong correlation with colour sensitivity. Hughes and Whitteridge (1973) could divide their analyzed on- or off-center units for the goat into transient and sustained units. Michael (1968a) who made an extensive study of units in the all-cone retina of the ground squarrel made no further classification of on- or off-center units.

Although many criteria can be employed, the presence or absence of a distinct sustained component in the cell firing during light-on in on-center cells or following light-off in off-center cells is the most used criterium for the classification. The various criteria for distinguishing on- or off-center units into two types show a high degree of correlation. Enroth-Cugell and Robson (1966) used the spatial linear summation criterium of center and surround for their classification into X- and Y-cells. Enroth-Cugell and Pinto (1972) distinguished surround-revealing and surround-concealing units, correlated with small and more extensive centers respectively. Winters et al. (1972) found two types of on-center units (Group I and Group II units) also based on the response of the periphery. Cleland et al. (1973) used a set of five criteria for their classification (viz. response to standing contrast, the periphery effect, the center size, responses to moving stimuli and to grating patterns). Using these criteria they found that the sustained units have not only a smaller center, but also a less extensive surround than transient units. The distinction was clear over a wide range of background intensities. Practically all units in the area centralis were sustained (90%), while beyond the area centralis 62.5% was sustained.

In this investigation a distinction will be made between the two types in the dark adapted state as well as in the light adapted state.

It will be shown that in the uniform center of the transient type of cells there is an overlap of a center and a surround mechanism (the elements of Rodieck and Stone's superposition model, 1965) while in the sustained type, evidence for a surround mechanism in the uniform center is lacking. Correlations of type with center size, maintained activity, location at the retina and sensitivity will be made. The results of mutual correlations will also be presented. It will be shown that units of one type have significantly smaller centers and that these units are located in a particular part of the retina. The significance and the function of these two types of units will be discussed. Finally, it will be shown that the quantum to spike ratios (QSRs) are very high in comparison with the QSRs of the cat, thus illustrating the phototopic function of the all-cone retina.

3.2. Methods

3.2.1. Preparation

Anaesthesia was induced in adult Tupaias (0.1 - 0.2 kg) with halothane in a mixture of 70% N_2O and 30% O_2. After an intramuscular injection of atropine sulphate (0.3 mg/kg) tracheotomy was performed. A tube provided with a smaller side-tube for sampling the expiration mixture was inserted into the trachea. This procedure has been used instead of the application of an endotracheal tube as described in chapter 1 and 2, because the field of view of the animal should be as large as possible. After this procedure the animal was placed in a stereotaxic apparatus and the head was fixed by pegs in the external ear canals. The outer end of the tube was connected with a ventilator (AIV, Amsterdam Infant Ventilator, Loosco) and the animal was ventilated with 60 strokes per minute. The side tube was connected with the Infant Capnograph (Godard) in order to measure the CO_2 percentage. Two electrodes were subcutaneously placed in a fore and a hind limb, and were used for ECG recording. In this phase the animal was paralyzed by an intramuscular injection of flaxedil (28 mg/kg hour) and the percentage halothane was diminished from values between 0.5 - 1.5 to 0.5. The heart rate ranged from 375 to 425 per minute, but was on the average about 390. A thermistor was placed in the rectum and the temperature of the animal was automatically kept at 37.5 ^{o}C by means of a heating blanket. The skin overlying the left optic tract was removed and a hole (5 x 3 mm) was drilled in the skull. In most experiments the dura was kept intact. All wounds were locally anaesthetized with a marcaine - adrenaline solution (0.5%). All pain contacts were locally anaesthetized with xylocaïne paste. During the preparation atropine was dropped into the eyes to dilate the pupils and to paralyze the lens muscles. Great care was taken to keep the eyes moist. After the preparation, the administration of halothane was stopped and the ventilation was adjusted at 5% CO_2 in the expiration mixture (chapter 1).

Phenylephrine hydrochloride (10%) was administered to the eyes to retract the eye lids and novesine (0.2%) was used to anaesthetize the eyes before mounting the contact lenses.

3.2.2. Contact lenses

Plano contact lenses (perspex) were used to keep the cornea moist. These lenses (diameter 7 mm) were provided with an artificial pupil (3 mm). To prevent corneal oedema in long lasting experiments the curvature of the central part (diameter 5.5 mm, $r = 4.4$ mm) was greater than that of the peripheral part.

Retinoscopy of the unaccomodated eye without lenses yields an apparent refractive error of 6 diopters. Probably this error is introduced because the inner surface of the retina is observed by this method and not the receptor layer. Glickstein and Millodot (1970) found that the refractive error increased as a function of the eye diameter in various mammals. Our value of 6 diopters agrees with their value from other animals with about the same eye diameter. With contact lenses the refractive error gives values of 13 diopters. So 7 diopters are introduced mainly by the different curvatures of the cornea surface and the contact lenses. This deviation was corrected for by spectacle lenses of 8 diopters, when the animal faced a white screen at a distance of 1 m.

3.2.3. Eye movements

Slow eye movements were measured in a separate experiment. A well fitting rubber contact lens with a mirror was attached to the corneal surface of the eye. A light beam was projected via the mirror onto the screen. Eye movements caused displacements of the spot on the screen (Fig. 3.1A). It was found that paralysis produced by flaxedil with (Fig. 3.1C) or without (Fig. 3.1B) d-tubocurarine was about equally effective in limiting the eye movements (maximally 0.3^o/ hour). About one hour after injection rapid eye movements occurred and could be induced by hand clapping. In these cases the spot disappeared for a moment and it was not possible to follow it. The heart beat and the ventilation respectively caused movements of 1' and 2'. The paralysis produced by using flaxedil alone was preferred, because the length of the experiment was shortened when d-tubocurarine was used, probably because of decreased blood pressure.

3.2.4. Stimulation

In most experiments spots were projected onto a screen by means of two hand controlled mirrors. In the later experiments a Maxwellian view device (Fig. 3.2) placed 12 cm before the eye, stimulated the eye. This optic device forms an image of D_1 or D_2 at a distance of 14 cm behind the eye; this 14 cm is necessary because of the refractive error. The image of D_3 is formed on a screen behind the device. Therefore, by removing the optic fibre between D_2 and D_3, it was possible to align the device with the pupil and the middle of the projected receptive field. By means of this device higher light intensities could be presented to the retina. A back-

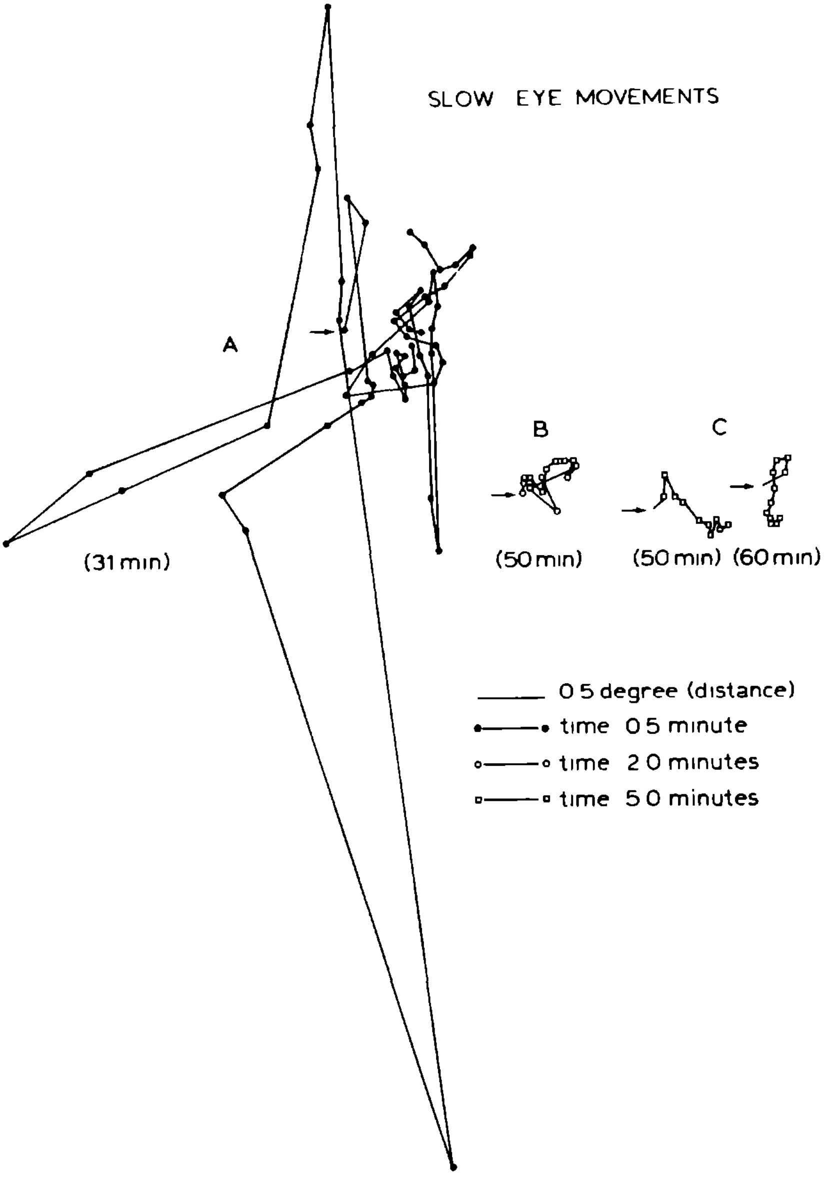

Figure 3.1 Slow eye movements under three different sets of conditions: A with halothane anaesthesia (2-2.5%)
B with flaxedil (28 mg/kg)
C with a mixture of flaxedil (10 mg/kg) and d-tubocurarine (2 mg/kg).
The total duration of the observations is indicated in brackets. Arrows indicate the beginning of the measurements.

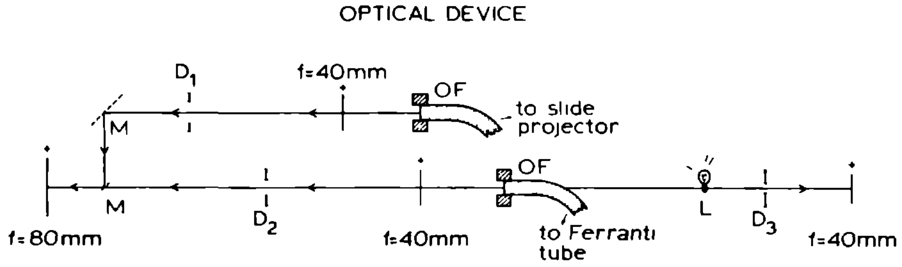

Figure 3.2 Schematic representation of the optical device. Images of D$_1$ and D$_2$ are formed on the retina; the image of D$_3$ is formed on a screen behind the device. O.F.: optic fibre; M: mirror (partially silvered; 50% transmission); L: lamp to direct the optical device between the eye and the projected receptive field on the screen when the optic fibres are removed. The focus (f) of the lenses is indicated.

ground field (20^{o}) was presented by means of a slide projector and a spot of variable size (0.3 - 9.6^{o}) by means of a cathode ray tube (Ferranti, CL64; maximum radiation output at 565 nm, half-amplitude values at 525 mm and 620 nm). Intensity adjustments were carried out by electronic control of the current through the tube (2.5 log unit) and by additional neutral density filters (Agfa-Gevaert). Symmetrical square wave pulses (modulation depth 99%) and short 10 msec pulses were presented with a frequency of 0.35 - 1 Hz by means of an oscillator (Wavetek). To search the units a tungsten filament tube in front of the eye flickering at a frequency of 4 Hz was used.

Light measurements were carried out with an AEG Luxmeter (UM). Intensities are expressed in equivalent quantal numbers of 550 nm/10 msec.degree^2 and the background intensities are expressed as the equivalent quantal number of 550 nm/sec.degree^2 upon the cornea and adapted to the effective pupil area. In these measures a correction for the position of the stimulus with respect to the eye axis is taken into account in the calculation of the pupil area. The spectral sensitivity curve of the eye of Tupaia measured by Tigges et al. (1967) by means of the ERG was used for the calculation of the quantal numbers.

3.2.5. Recording technique

Tungsten microelectrodes were used to record spikes from optic tract fibres. These electrodes were electrolytically sharpened using the method of Hubel (1957) and were coated 3 times with Isonel 31. After this procedure the varnished tip of the electrode was exposed by briefly applying a voltage of 3 V (electrode negative) to the electrode which was placed in saline. Under continuous inspection the appearance of bubbles from the tip was observed with a light microscope (100 x) indicating that the electrode was 'open'. In most cases the resistance decreased then to 0.5 - 2.5 MΩ at 1000 Hz. Tip diameters ranged up to 1 μ and the diameter of the electrode at a distance of 40 μ from the tip was about 7 μ. With these electrodes penetrations through the dura are possible and the resulting recordings were successful. Practically no units were found when micropipets were used possibly because of the small diameters of the fibres (1 - 2 μ) and the small receptive areas of the electrodes.

After amplification (Grass P16), spikes were made visible on an oscilloscope and made audible via a loudspeaker. Furthermore the spikes were fed into a window discriminator and converted into standard pulses.

The stability of the recordings was generally sufficient for the complete program of stimulation conditions which lasted for 180 minutes. Most of the units discussed in this chapter have been completely investigated.

3.2.6. Data analysis

In most cases the same stimulus was presented thirty times. The choice of thirty presentations is a compromise between the accuracy needed to observe a response and the number of different stimuli which can be presented during the time that recordings can be made from a single unit. The time of onset of the occurrence of spikes after the beginning of a stimulus was stored with the aid of a computer (PDP-9). Also other parameters such as animal number, unit number, intensity, and frequency of presentation of the stimulus, modulation depth, type of stimulus and spatial parameters, such as diameter of the stimulating spot, and the background field were automatically stored. Because of the on-line connection with the PDP-9 we were able to follow the construction of a dot-display on a scope during the recording. A Post-Stimulus-Time-Histogram (PSTH) with a bin width of 5 msec and the data calculated from such a PSTH were also presented. Unless specified the ordinate of the PSTHs is expressed in spikes/sec.bin.

Important data such as the number of spikes in the burst, the maintained activity (expressed in numbers of spikes) during a period as long as the burst, the maximum firing rate and the time of onset of the maximum firing rate were calculated. Also the number of spikes in the first 100 msec after the onset of the stimulus and the number of spikes in the last 100 msec preceding the stimulus were calculated.

The burst duration was calculated by subtracting the 'latency of the burst' from the 'time from the onset of the stimulus to the end of the burst'. These two times were measured by first calculating the mean (m) and the standard deviation (s) of the number of spikes over the last 40 bins of the PSTH preceding the onset of the stimulus. The criterium was m + 2s (Fig. 3.3). The 'latency of the burst' was indicated by the first bin before the maximum of the PSTH, whose number of spikes exceeded the criterium; the end of the burst was indicated by the first bin after the maximum of the PSTH, whose number of spikes was below the value of the criterium. In sustained units the 'time from the onset of the stimulus to the end of the burst' was never found during light-on, because the activity during light-on was always higher than the activity during light-off; in these cases this parameter had to be estimated from the PSTH manually.

The number of extra spikes in the burst can be calculated in two ways. 1° By subtracting the maintained activity (during the burst) from the total number of spikes in the burst itself. In this case the calculated number is always a little too low because of the definition of the burst duration. 2° By subtraction of the maintained activity (100 msec) from the total number of spikes (100 msec), when the end of the burst is within this period. In this case the calculated number is only too low when the burst is followed by suppression. The differences between these two methods are slight and not significant (see chapter 5). The calculation according to method 2 is followed in this chapter.

The number of extra spikes has been taken as a measure of the response to short pusses, rather than the maximum firing rate. This was done because it is then possible to

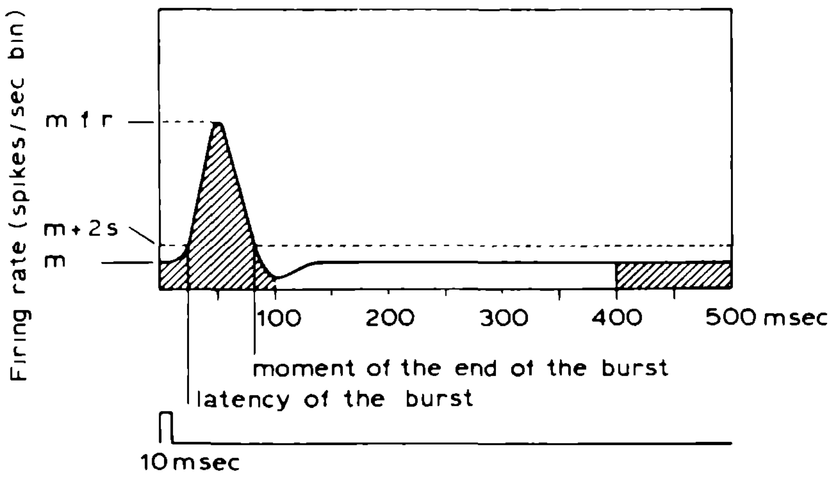

Figure 3.3 Characteristics of a Post-Stimulus-Time-Histogram (PSTH) calculated. The response to a short (10 msec) pulse is shown. M.f.r.: maximum firing rate; m: maintained activity; s: standard deviation of the maintained activity. Hatched areas: periods of 100 msec from which the number of spikes have been calculated.

calculate the quantum to spike ratio, which is an excellent and important parameter for defining the sensitivity (Barlow and Levick, 1969b). There are also other reasons: the maximum firing rate, for instance, represents the number of spikes in one bin in the PSTH and is therefore a more fluctuating quantity than the total number of spikes in a burst; furthermore one must be careful when the maximum firing rate is used because it is not always correlated with a same number of extra spikes: it will be shown that in the light adaptad state the burst duration is shorter than in the dark adapted state and that burst duration is different for the two types of units found. The period of calculation (100 msec) of the number of extra spikes in the burst is, for most units, long enough when short 10 msec pulses are used. In some sustained units, however, the burst duration is somewhat longer, but the contribution to the total number of extra spikes is seldom more than 20%. The optimal summation time (Barlow and Levick, 1969b) could not be consistently defined with our data.

3.2.7. Definition of terms

The quantum to spike ratio (QSR) is the ratio of the number of equivalent quanta at 550 nm incident on the cornea and the number of extra spikes during the first 100 msec after the onset of a 10 msec pulse. Although there is some uncertainty concerning this ratio at very low stimulus levels, it was found that it was, on the average, constant up to 4 or 5 extra spikes. At higher response levels saturation occurs and the QSR increases significantly (see also Discussion).

The terms 'activation' and 'suppression' will be used for respectively an observable increase or decrease of the number of spikes with respect to the maintained activity. The corresponding terms 'excitation' and 'inhibition' will be reserved for the model.

The results will be discussed in terms of the same elements, which Rodieck and Stone (1965) used in their superposition model, viz. a 'center-mechanism' and a 'surround mechanism'. The center mechanism is the process which predominates in the center of the receptive field. In on-center units this mechanism causes the cell to increase its firing rate when the light is on and to decrease its firing rate when the light is off. The surround mechanism of on-center units causes a decrease in the firing rate when the light is on and an increase in the firing rate when the light is off. It will be shown in this chapter that the spatial arrangement of this antagonistic surround mechanism is different for the two types of on-center units. Besides the term 'center' the term 'uniform center' (Cleland and Enroth-Cugell, 1968) will also be used; this refers to that region in which Ricco's law holds. The region outside the uniform center will be called the receptive field 'periphery'.

3.3. Results

3.3.1. The size of the uniform center

In order to determine the linear summation area (uniform center) we used concentric discs of light and measured the QSR. The uniform center is the area in which the QSR stays constant. A stimulus time of 10 msec was used. The response is characterized by an increase in the number of spikes, whether or not followed by a suppression (Fig. 3.4).

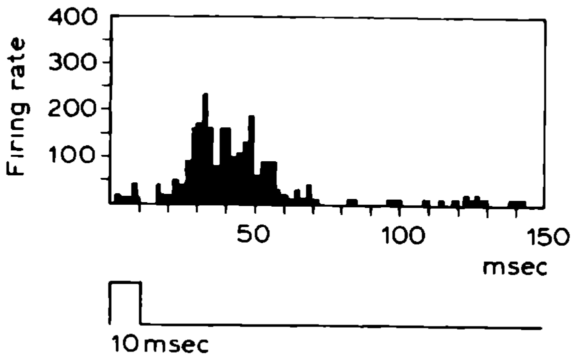

Figure 3.4 Post-Stimulus-Time-Histogram (PSTH) with pulse stimulation. Bin width: 2 msec. Firing rate in spikes/sec.

Stimulus response curves were determined for different stimulus areas. In Fig. 3.5 such curves have been plotted for one unit. It can be seen that the lower part of the curves approximates to a straight line. The slope of this line gives the reciprocal of the QSR. In this example it is clear that the QSR does not change significantly up to a diameter of 1.2°. Above this values the QSR increases. If a part of the stimulus area extends beyond the center then the number of quanta falling outside the center will contribute less, or even not at all to the extra spikes; the number of quanta increases with stimulus area and the number of spikes will tend to become constant in this case, provided that the surround mechanism in the periphery has no influence. Therefore the QSR will be proportional to the stimulus area if the stimulus exceeds the center area. In a double logarithmic plot of QSR against stimulus area the curve starts as a horizontal line followed by a transitional region and then becomes a straight line with the expected unity slope (Fig. 3.6). The gradual transition can be explained by a lower sensitivity at the border of the center and by the often observed elliptical form of the center. In the cat elliptical field centers are also more common than circular ones (Fukuda, 1971). In the area-QSR graph the area value at 1.25 times the horizontal QSR value was used to calculate the diameter of the uniform center (Fig. 3.6). In this way the diameter of 38 on-center units were measured (Table 3.1).

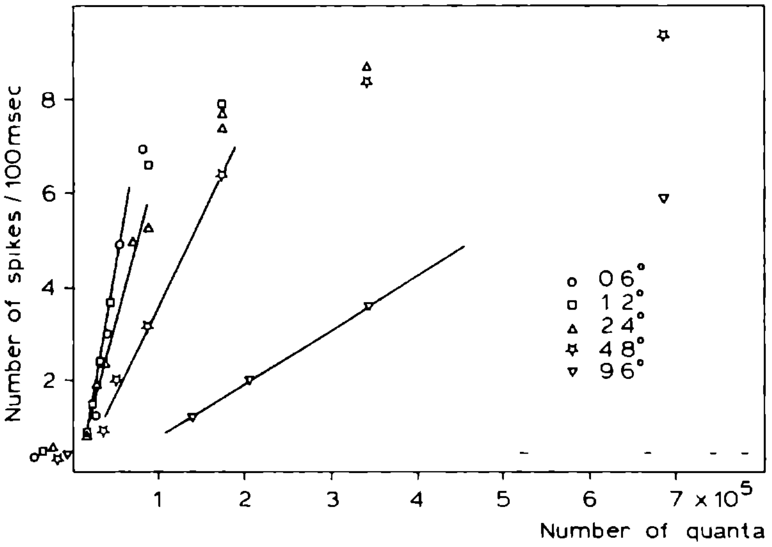

Figure 3.5 Stimulus response curves in the dark adapted state for different stimulus diameters. The broken line indicates the mean level of maintained activity in the dark; at the left of the ordinate the various values of this activity are indicated. Pulse duration: 10 msec. Unit 50-0 (transient).

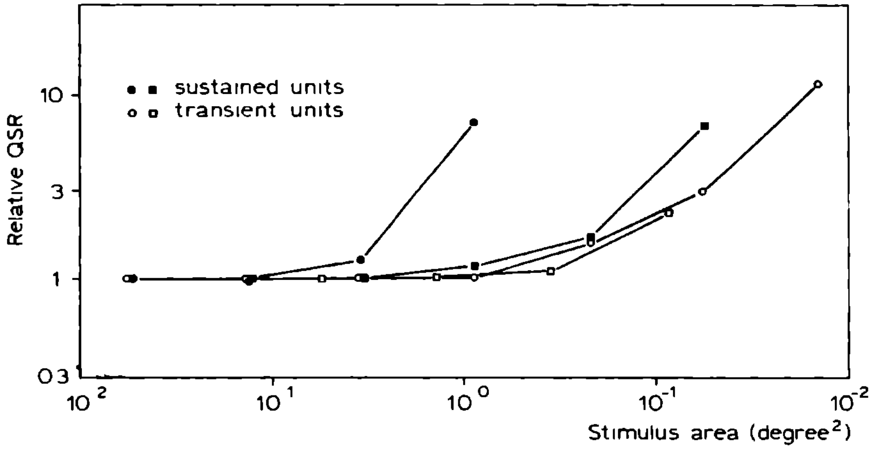

Figure 3.6 Area-QSR (quantum to spike ratio) curves for different units.

Diameter of the uniform center	Number of units
0.5 - 1°	8
1 - 2°	14
2 - 4°	16

Table 3.1 Uniform center sizes of 38 on-center units.

3.3.2. Characterization of on-center units based on the dynamic aspects of the response

3.3.2.1. Responses to long square wave pulses

On considering the responses to long square wave pulses presented to the uniform center, the units can be divided in two distinct groups (Fig. 3.7A and B). One group responded with a short transient burst followed by a practically unchanged level of steady state activity during light-on. As can be seen in Fig. 3.7A there is often a short period of suppression followed by a smaller burst during light-off. This characteristic of the response is not always present in this group of units and is dependent on the intensity of the stimulus. The presence of the burst at light-off is an indication that the surround mechanism is relatively strong in comparison with the center mechanism inside the uniform center. The other group responded with a longer burst followed by an increased steady state activity during light-on (Fig. 3.7B).

Two aspects of these responses will now be considered, viz. the level of steady state activity with regard to the maintained activity in the dark and the burst duration. The maintained activity in the dark during a period of 100 msec was subtracted from the mean activity during the last 100 msec of the stimulus-on period. Thirty units were handled in this way.

In Fig. 3.8 the relation of the number of extra spikes of the maintained activity to the maximum firing rate is plotted. It can be seen that two distinct groups exist. In one group the response in the steady state is hardly increased with respect to the maintained activity in the dark and is about the same over a wide range of maximum firing rates. This group will be called the transient group. In the other

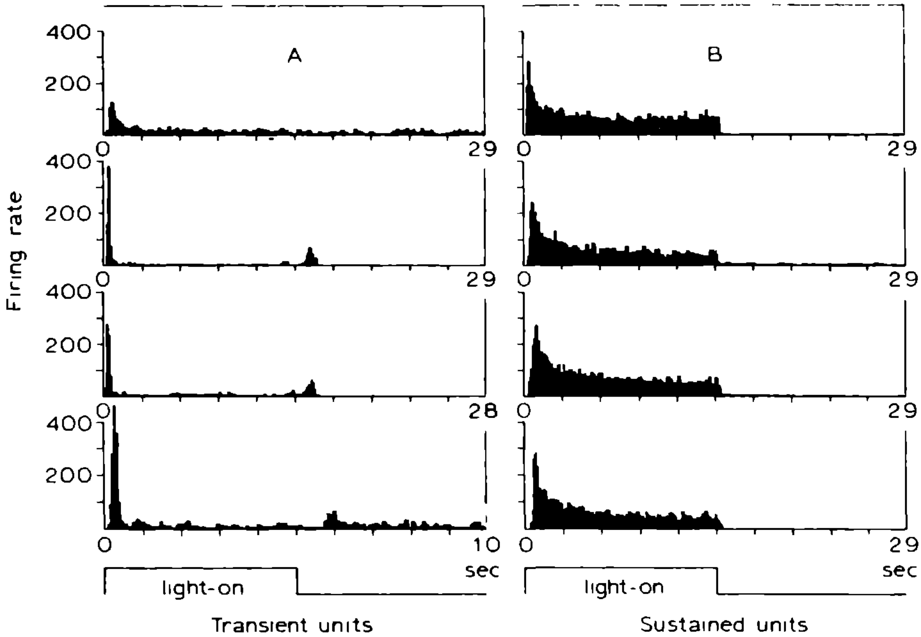

Figure 3.7 PSTHs of 4 transient (A) and 4 sustained units (B). Stimulation applied to the uniform center.

group the response in the steady state is increased and gives a small positive correlation with the maximum firing rate. This group will be called the sustained group.

In Fig. 3.9 the maximum firing rate and the burst duration are plotted against each other for the same units. The total length of the burst was chosen and not e.g. the width at half-amplitude values, because the difference between the two groups is much clearer, when the lowest part of the burst in the PSTH is considered. Again a sharp distinction can be made. The sustained group consists of units giving a burst longer than 150 msec; the transient group consists of units giving a burst shorter than 150 msec. In transient units two subgroups can be distinguished, one with a burst duration of about 100 msec and one with a burst duration shorter than 60 msec. Fig. 3.10 shows that what is happening. Transient units belonging to the middle group of Fig. 3.9 give responses as shown in Fig. 3.10A and B. The other transient units responded in the manner shown in Fig. 3.10C. Suppression can affect the burst during light-on and causes a shortening of the burst duration. It is always correlated with a smaller burst during light-off.

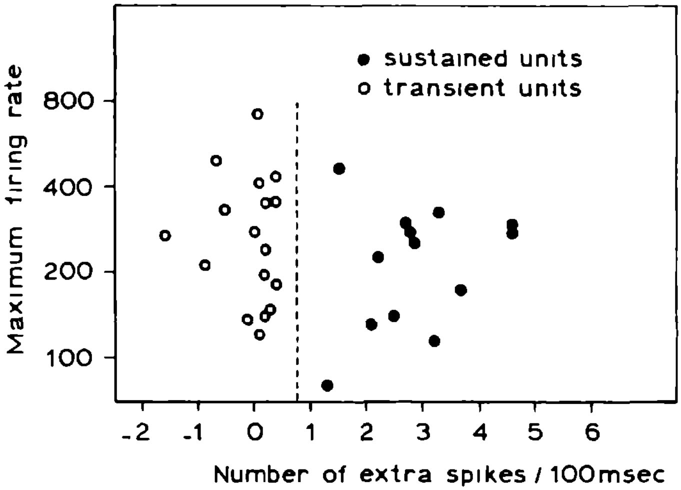

Figure 3.8 Relationship between the maximum firing rate and the number of extra spikes as a response to long square wave pulses of various units (30). Number of extra spikes = number of spikes during the last 100 msec before light offset minus number of spikes during a 100 msec period in the dark adapted state. Stimulation applied to the uniform center.

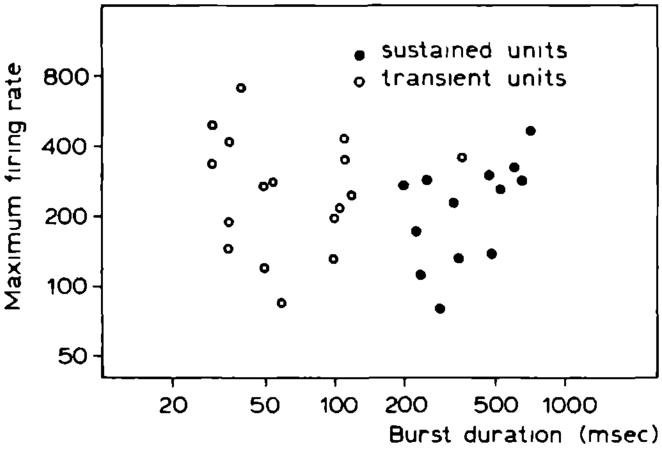

Figure 3.9 Relationship between the maximum firing rate and the burst duration as a response to long square wave stimulation of various units (30). Stimulation applied to the uniform center.

The time of occurrence of the suppression during light-on and the time of occurrence of the activation during light-off are of the same order (60-80 msec). This is an indication that the latency of the surround mechanism is longer than the latency of the center mechanism or, alternatively, that an apparent latency difference is caused by the slower building up of the inhibition of the surround mechanism with respect to the excitation by the center mechanism.

Besides a lack of suppression during light-on and an activation during light-off in sustained units, an increased steady state activity was also observed. Furthermore, the activity during light-off was much more suppressed in

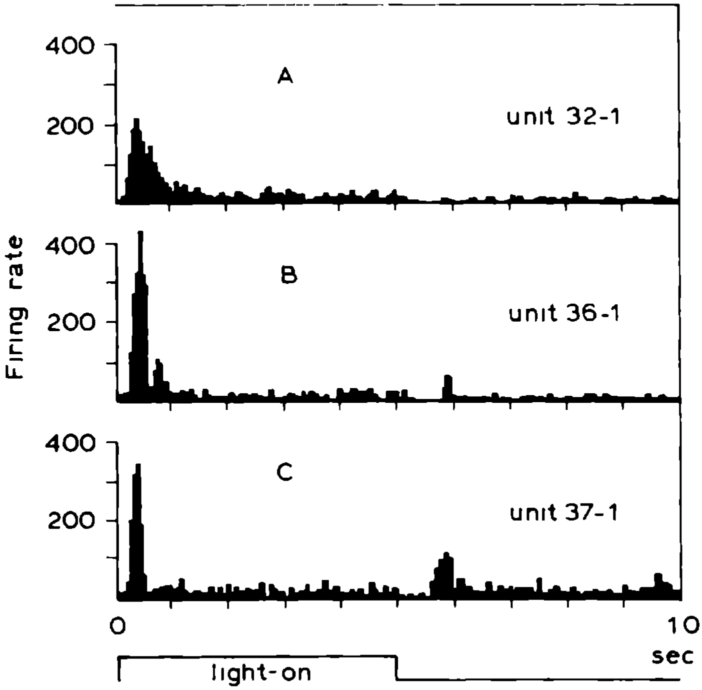

Figure 3.10 PSTHs of transient units as responses to long square wave stimulation. In A and B the burst duration is longer than in C. In B suppression tends to shorten the burst. Activation following a suppression at the offset of light is present in B and C. Stimulation applied to the uniform center.

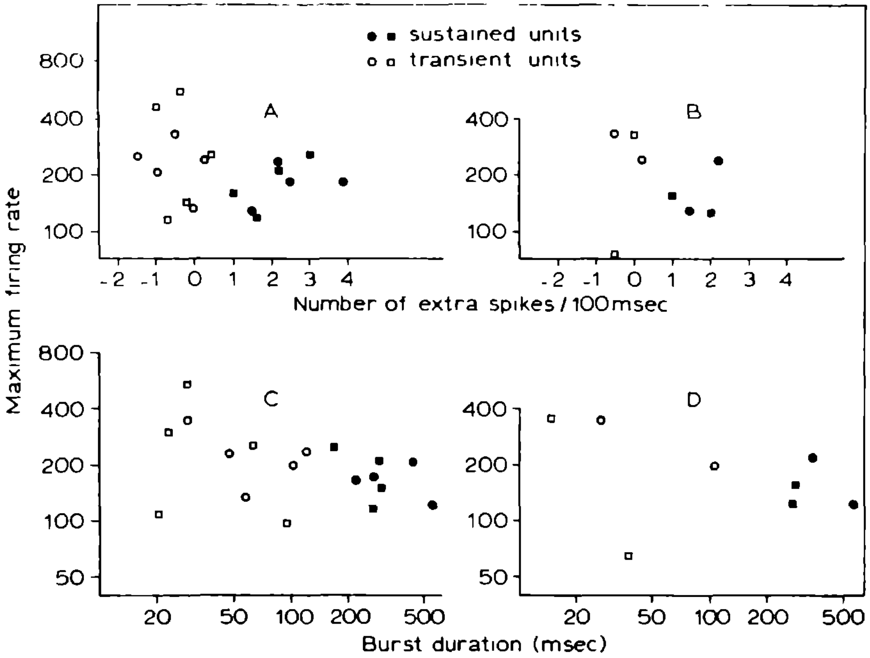

Figure 3.11 Comparison of the 'number of extra spikes' (A and B) and the burst duration (C and D) in the dark (circles) and in the light adapted (squares) state. The sensitivity is decreased by light adaptation (A and C: 0.8 - 1.5 log units; B and D: 2 log units). The units present in B and D are also present in A and C. Long square wave stimulation applied to the uniform center.

sustained than in transient units (Fig. 3.7A)and B). This is also an indication that the surround mechanism is weak or absent in the uniform center of sustained units.

Also in the light adapted state the difference between sustained and transient units remains clear. No difference in the number of extra spikes during the steady state in the dark and light adapted state can be observed (Fig. 3.11A and B). However, the burst duration becomes shorter with light adaptation (Fig. 3.11 C and D), but the difference between the two types remains. These results are in agreement with the results of Cleland et al. (1973), who found that, in the cat, the distinction between sustained and transient units survived over a wide range of background intensities.

3.3.2.2. Responses to short pulses

The results discussed in the previous section show that the burst duration was shorter in transient units than in sustained units when long square wave pulses were presented. In this section the results of stimulation with short 10 msec pulses will be presented. For each unit the burst duration was plotted against the number of extra spikes during the first 100 msec after onset of the stimulus.

In some transient units (Fig. 3.12A and B) the burst duration increases with the number of extra spikes or remains the same, while in other transient units the burst duration decreases. In the last mentioned group the responses to long square wave pulses always show a small burst during light-off, while this is absent in the former. It is possible that the surround mechanisms shortened the burst.

The sustained units never showed any decrease in the

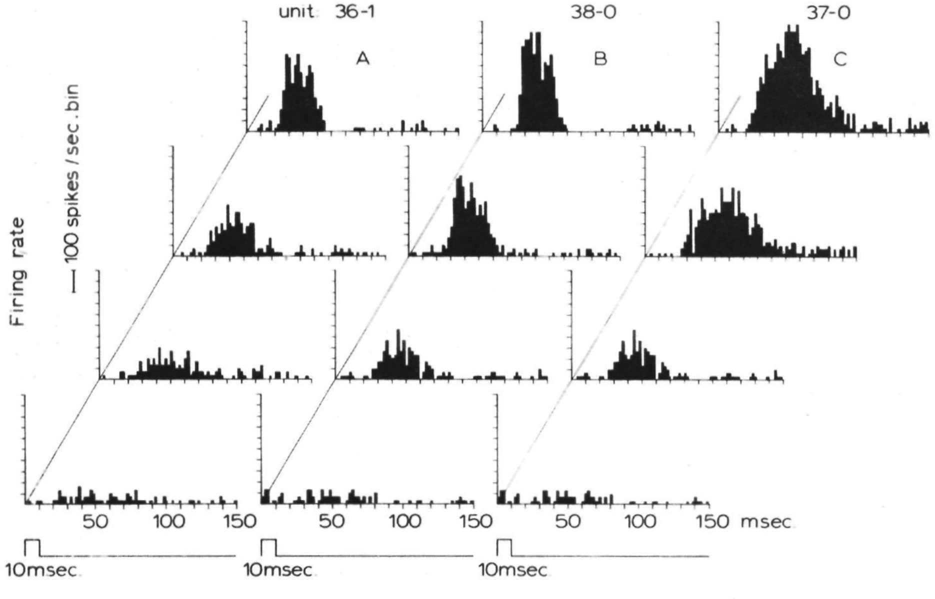

Figure 3.12 PSTHs of responses to short (10 msec) pulses inside the uniform center with increasing stimulus intensity. 36-1 and 38-0: transient units; 37-0: sustained unit. Bin width: 2 msec.

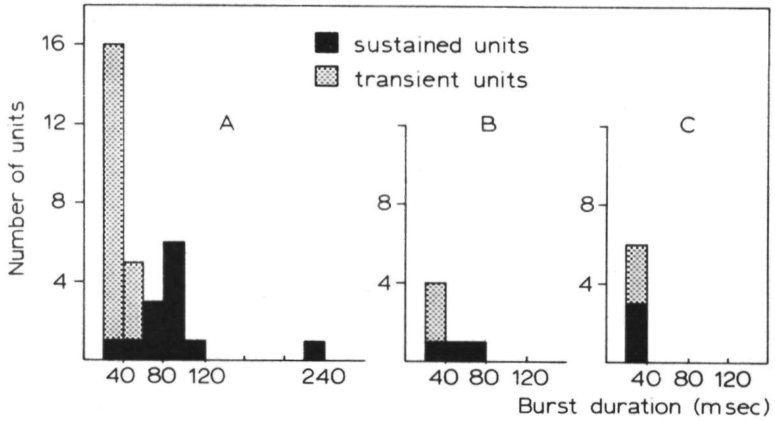

Figure 3.13 Histograms of the burst duration at short 10 msec pulses applied to the uniform center. A: dark adapted, B: light adapted (sensitivity decrease: 0.4 - 0.7 log units). C: light adapted (sensitivity decrease: 0.8 - 1.5 log units). The burst duration was calculated using as a criterium 5 extra spikes in the burst.

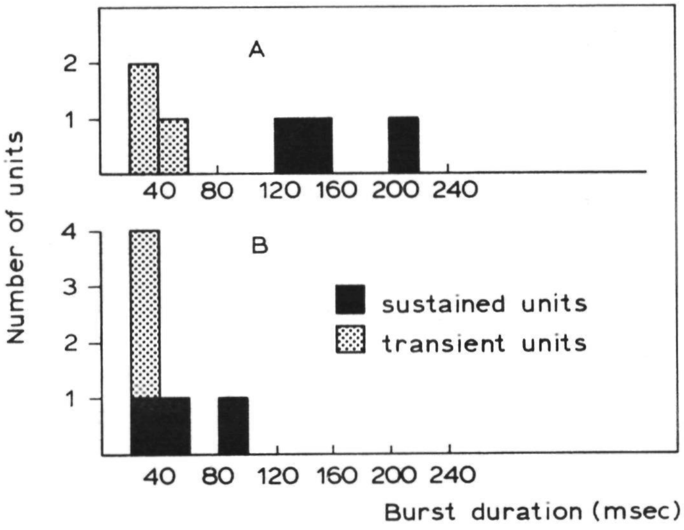

Figure 3.14 Histograms of the burst duration as response to short (10 msec) pulses. A: stimulation applied to the uniform center, B: diffuse stimulation.

burst duration as the number of extra spikes in the burst increased (Fig. 3.12C). Here the surround mechanism was not observable when long square wave pulses were used. Fig. 3.13A shows that the difference between the two types is marked in the dark adapted state. All transient units give a burst shorter than 60 msec and most sustained units give a burst longer than 60 msec. There is a slight overlap of only two units (total 32 units).

In the light adapted state the burst duration especially of the sustained units decreases, as is the case with long square wave pulses. From Fig. 3.13B and C it can be seen that the difference between the two types diminishes and disappears with increasing light adaptation.

Up to now only responses to short pulses presented inside the uniform center have been considered. We will now consider the burst duration of the response to diffuse stimulation in the dark adapted state for both types of units and will compare these with the burst duration resulting from center stimulation. Fig. 3.14 shows that the burst duration remains almost unchanged in transient units whether a diffuse stimulus (B) or a stimulus inside the uniform center (A) is presented. In sustained units, however, a diffuse stimulus shortens the burst in comparison with stimuli presented inside the uniform center. The burst duration becomes as short as in transient units. These results may indicate that in the receptive field periphery of sustained units a surround mechanism is dominating, while in the receptive field periphery of transient units both a center and a surround mechanism are present, which is similar to the case of a uniform center.

3.3.3. Maintained activity in the dark, uniform center size, location at the retina, sensitivity of the two types of units and mutual correlations

3.3.3.1. Maintained activity in the dark

Maintained activity in the dark was measured for the two types. None of these units was completely inactive in the dark, but most units had a low activity (Fig. 3.15). There is not correlation between this activity and the type of unit. The literature contains no data between type of units and dark activity. More details about the maintained activity in the light adaptad state will be presented in the next chapter.

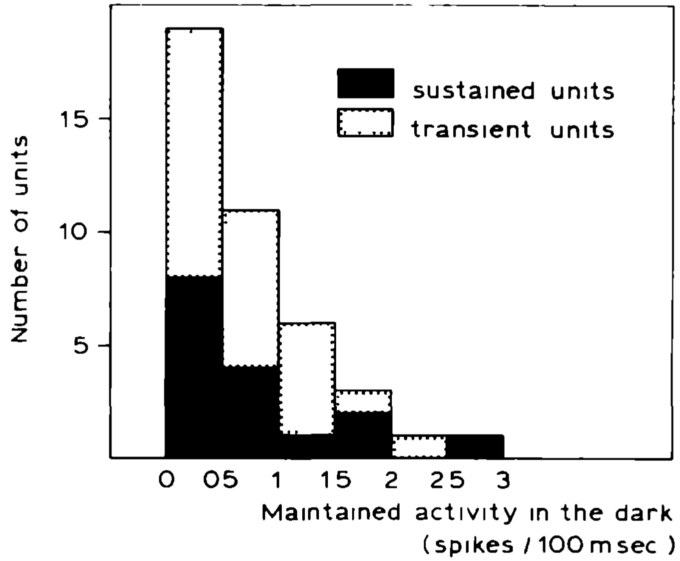

Figure 3.15 Histogram of the maintained activity in the dark (41 units).

3.3.3.2. Uniform center size

As mentioned in paragraph 3.3.1. all units were divided into three groups with regard to the uniform center size. In table 3.2 the uniform center size is shown for both types of units.

Diameter of the uniform center	Number of sustained units	Number of transient units
0.5 - 1°	8	0
1 - 2°	5	9
2 - 4°	4	12

Table 3.2 Uniform center sizes of sustained and transient units.

It is clear that units with a small center tend to be sustained units. In this respect the results are in accordance with those of Cleland et al. (1973) and Ikeda and Wright (1972).

There is no correlation between the uniform center size and the maintained activity in the dark.

From the area-QSR curves (Fig. 3.6) it is apparent that the slope of the transitional part is steeper for sustained than for transient units. As a measure of the transitional part, the points on the area-QSR curve where the QSR was increased by a factor of 2 and 1.5 were first determined; next, the ratio of the areas relating to these points (A2 and A1.5) was calculated. In this way a factor was obtained for each unit indicating a fixed sensitivity decrease (1.33) at an area with respect to A1.5. Table 3.3 shows the results.

$\mathrm{Log}\ \frac{A2}{A1.5}$	Number of sustained units	Number of transient units
0.12-0.24	6	3
0.24-0.36	4	7
0.36-0.48	-	1
0.48-0.60	-	1

Table 3.3 Transitional parts in log units for sustained and transient units.

Although the two distribution of the ratios overlap, the ratios of sustained units are significantly lower ($P < 0.01$, test of Wilcoxon). In table 3.4 the distance between the borders of A2 and A1.5 is expressed in degrees and it appears that the difference between the two types is even more pronounced, because centers of sustained units are, on the average, smaller than those of transient units (cf. Table 3.2).

Distance	Number of sustained units	Number of transient units
0.0-0.2°	6	3
0.2-0.4°	4	3
0.4-0.6°	-	5
0.6-0.8°	-	1

Table 3.4 Distance from the border of A1.5 to the border of A2 for sustained and transient units.

Ikeda and Wright (1972) have measured the sensitivity gradients of sustained and transient units in the cat. The mean slope of the gradients of sustained units (diameter on a linear and sensitivity on a logarithmic axis) was 10 times that of transient units. The distinction they made between boundary cells and expansion cells is based on these sensitivity gradients. The difference they found between the two types was more distinct than we observed since they worked with spots passing through the receptive fields, while we used concentric discs of light. Furthermore they worked with sustained units which had extremely small (0.2-0.4°) centers.

3.3.3.3. Location at the retina

The retina of the *Tupaia* can be divided into four quadrants with respect to the eye-axis. The image of objects in front of the animal are formed in the temporal-superior quadrant because *Tupaia* has laterally directed eyes (Fig. 3.16) and because the head of the animal is directed a

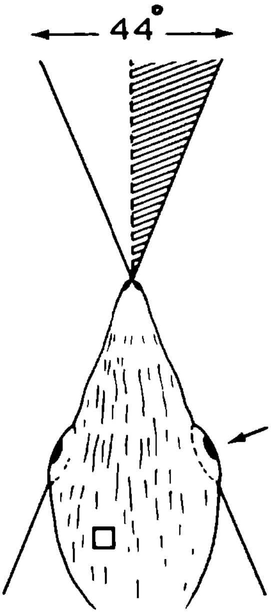

Figure 3.16 Dorsal view of the head of *Tupaia*. The region of binocular vision is indicated and measures 44°. The arrow indicates the direction of the eye-axis in the experiments. Hatched area: part of the visual field where most ipsilateral units are present. Square: recording place.

little downward due to the stereotaxic method used (the plane passing through the centers of the external auditory meati and the lower margins of the orbital opening was set horizontally, cf. Tigges and Shantha, 1969). Most units were found to be present in this temporal-superior quadrant.

From Fig. 3.17 it can be seen that no units were found in a circular part of the temporal-superior quadrant. Around this region the majority of cells is of the sustained type, while still more peripherally preponderantly transient units are present. It is probable that the region surrounded by sustained units only represents the area centralis, because

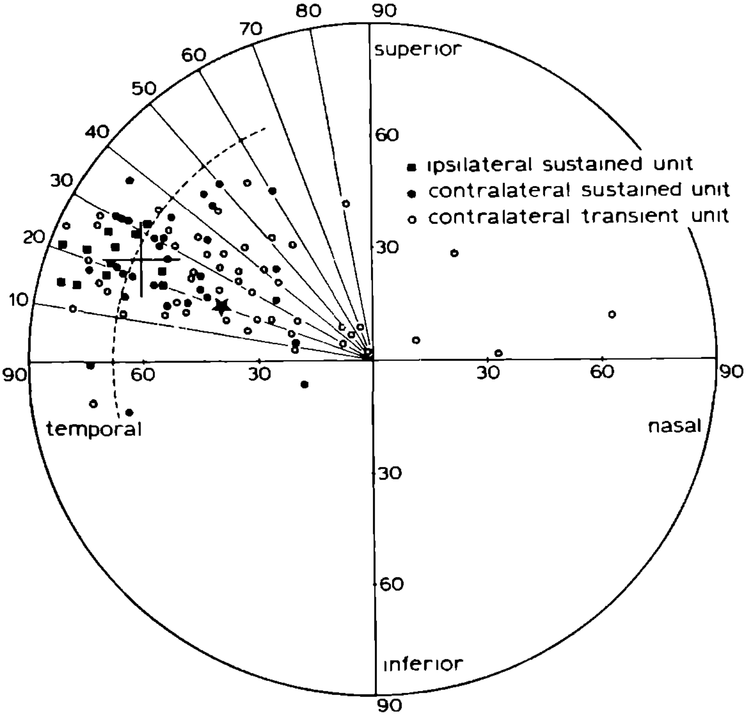

Figure 3.17 Projection at the retina. Closed symbols: sustained units. Open symbols: transient units. Squares: ipsilateral sustained units. Cross: area centralis. Star-symbol: optic disc. Broken line: central axis of the binocular visual field (cf. Fig. 3.16).

the size of this region and the distance of it from the optic disc are in agreement with the results of Castenholz (1965). A high concentration of ganglion cells (5 times more than in the nasal region) and an increased density of receptor cells and bipolars is present in this area. Furthermore it appeared from our experiments that this region is situated on the central axis of the binocular visual field as will be shown below. The lack of units in the *area centralis* in our experiments is possibly caused by sampling bias brought about by the nature of the electrode. In the cat fibres originating from the *area centralis* are thinner than fibres originating elsewhere in the retina (Stone and Höllander, 1971) and it is possible that the thinnest fibres of the sustained group with the smallest receptive fields are present in the *area centralis* of *Tupaia*.

Thirteen out of 91 units were ipsilateral and sustained. This finding is in agreement with the anatomical evidence (Laemle, 1968), viz. that about 10% of the fibres do not decussate. Furthermore all units in the ipsilateral eye were present in the binocular visual field close to the *area centralis* and almost all units were located in a special part of this field (Fig. 3.16).

The clear correlation between type and location on the retina also means that there is a correlation between the location of the units and the size of the uniform center. Within one group (sustained and transient) no significant correlation is found between center size and eccentricity on the retina. The maintained activity in the dark is also not correlated with the location at the retina.

3.3.3.4. Sensitivity

The sensitivity of the units was measured in terms of QSR. There is a wide range of QSR values: from 10^3 to 10^7 (Fig. 3.18). The transient units are about as sensitive as the sustained units in the average.

Because general condition and transparency of the optical media can each play an important role in experiments of long duration, the sensitivity and the uniform center size were tested as a function of the time after the beginning of the experiment. It appeared that all units found in the beginning of the experiments were on the average as

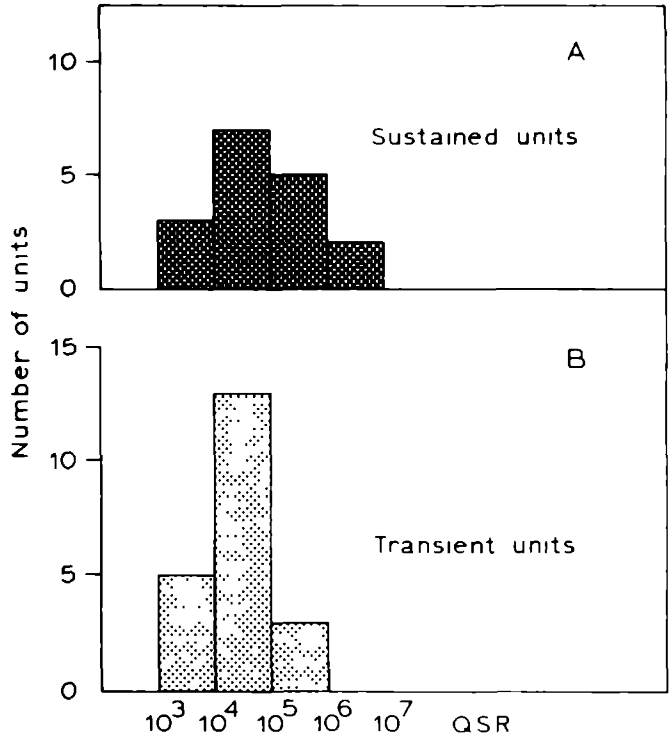

Figure 3.18 Histograms of the Quantum to Spike Ratios (QSRs) for sustained (A) and transient units (B).

sensitive as units found later on. No significant difference between the mean size in the beginning and at the end of the experiments was found. Thus transparency changes during the experiment which would influence the measured center size probably did not occur. So the wide range of QSR cannot be attributed to transparency differences of the optical media during the experiments.

No correlation was found between sensitivity on the one hand and center size or maintained activity in the dark or location at the retina on the other hand.

Under optimal experimental condition in the cat, a QSR of 2-14 has been found for units which were backed by the tapetum lucidum (Barlow et al., 1971). Our QSRs range from 10^3 to 10^7. It is clear that the rod system in the cat as compared to the cone system in Tupaia is responsible for this difference, which is in agreement with the different modes of life of these two animals.

3.4. Discussion

The results reported in this chapter can be understood in terms of a model in which two mechanisms are present, viz. a center mechanism and a surround mechanism, the same elements as in Rodieck and Stone's (1965) superposition model (see also Büttner and Grüsser, 1968). The spatial arrangement of these two mechanisms on the retina is different for the two types in *Tupaia* (Fig. 3.19). The center mechanism of on-center units is excited at the onset of light and inhibited at the off-set of light. The surround mechanism is antagonistic and is excited at the offset of light and inhibited at the onset of light. It is thought that the surround mechanism is inhibitory with respect to the center mechanism. An essential characteristic of the surround mechanism is the longer latency (60-80 msec).

In the sustained units no surround mechanism was observable in the uniform center. However, the possibility exists that this mechanism makes a slight contribution in the uniform center. The model of a sustained unit shows that the surround mechanism is more extensive at the outer border as is the case in transient units (Fig. 3.19, left part). Although this is difficult to prove, the general picture is clear: in sustained units the surround mechanism dominates much more in the peripheral part of the field than in transient units. It will be shown in the next chapter that an antagonistic response can be isolated in the periphery of sustained units and not in transient units. The model explains the following results with long square wave stimulation at the uniform center (a in Fig. 3.19):

1° The high steady state activity in sustained units and the hardly increased steady activity in transient units (3.3.2.1).

2° The strong suppression in sustained units and the practically unchanged activity in transient units during light-off (3.3.2.1).

3° The long burst duration in sustained units and the short burst duration in transient units (3.3.2.1 and 3.3.2.2).

4° The suppression after the burst at the onset of light and the smaller burst after the suppression at the offset of light in transient units, and the lack of this pattern in sustained units (3.3.2.1).

The model also explains:

5° The shorter burst duration of sustained units on diffuse stimulation (b in Fig. 3.19). The duration of this

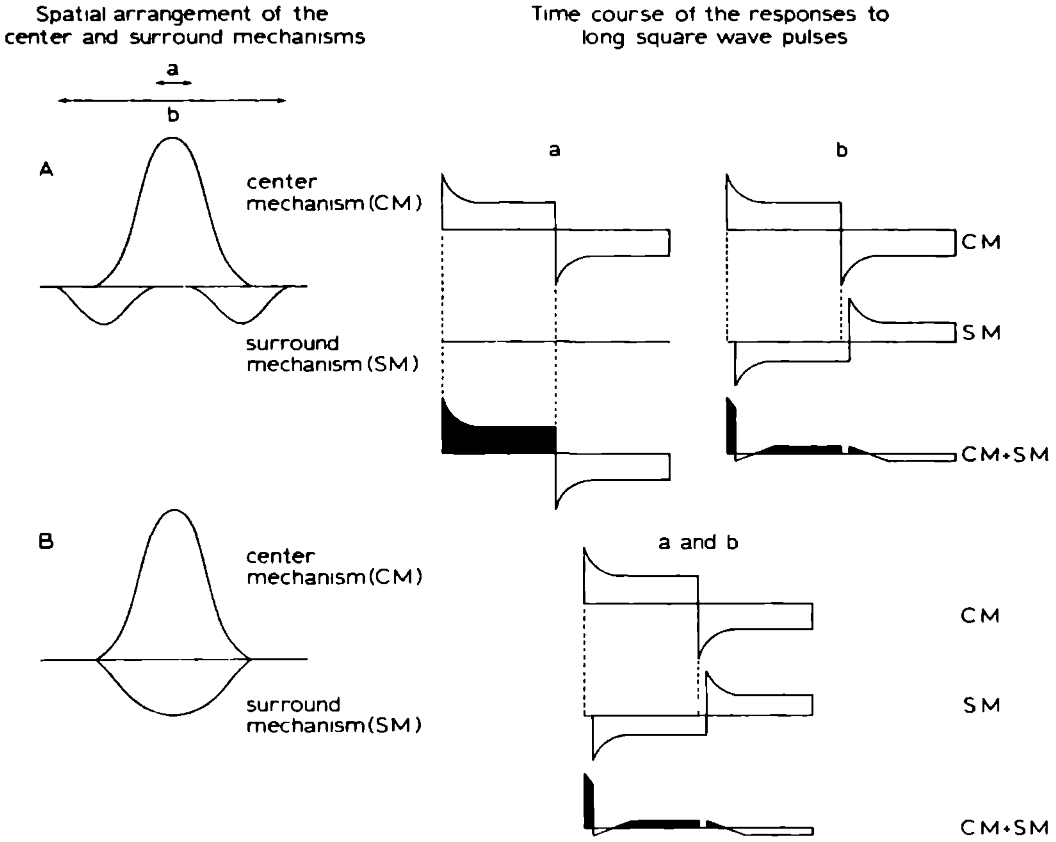

Figure 3.19 The model. The spatial arrangement of the center and surround mechanism for sustained (A) and transient units (B) is shown at the left. The dome shaped curves depict the strength of the mechanisms at different retinal positions. The time courses of both mechanisms for center (a) and diffuse (b) stimulation are shown at the right. C.M.: center mechanism; S.M.: surround mechanism.

burst remains the same in transient units (3.3.3.2).

6° The steeper sensitivity gradient in the area-QSR curves of sustained units (3.3.3.2).

This model does not explain the horizontal parts in the area-QSR curves and the more extensive uniform centers in transient units.

The time course of both the center and the surround mechanism is at first transient, followed by a level of steady state activity (Fig. 3.19, right part). An alternative time course might consist of a level of steady state activity only. The latter time course is present in the cat when there is no light adaptation (Enroth-Cugell and Shaply, 1973). In Tupaia this time course cannot explain the suppression following the activation during light-on and the reverse during lifht-off.

Winters et al. (1973) have found in their studies concerning the receptive field periphery of on-center units in the cat two types of cells which they term group I and group II cells. In the cells of group I it is not possible to isolate the off-response by annuli different in distance from the receptive field center. This, however, is possible in the other type, group II cells. Hickey et al. (1973) argued that group I cells probably correspond with transient cells and group II cells with sustained cells. Their group I cells have in the center a strong surround mechanism and in the periphery a strong center mechanism; the center and surround mechanism in group II cells are spatially far better separated with weak or totally absent antagonistic mechanisms in the uniform center and the periphery. Our results concerning the two mechanisms for Tupaia are in agreement with their model.

The better spatial separation of the two mechanisms in sustained units is in accordance with the steeper slopes of the transitional parts of the area-QSR curves. But there is a considerable overlap for the two types (3.3.3.2), which can be accounted for by the frequently observed elliptical form of the center when working with concentric stimulus spots.

In Table 3.5 the diameter of receptive field centers as measured by different authors are presented. From this table it becomes clear that recording from fibres yields a larger mean diameter than recording from ganglion cells, because when recording from fibres receptive fields from the whole retina are involved. Our population of sustained units has about the same mean diameter and standard deviation as the sample of units in the cat measured by Enroth-Cugell and Robson (1966). Approximately the same experimental conditions were employed: recording from the optic tract and sampling in a wide range of retinal eccentricities. In the cat the center size increases with retinal eccentricity (Fischer and May, 1970), but in the ground squirrel (Michael, 1968a) no correlation was found. In Tupaia, however, sustained units were found in the area centralis, as in the cat, and elsewhere preponderantly transient units were found. So also in Tupaia a rough correlation exists: units in or directly beyond the area centralis are sustained and small, while more peripherally the units are transient and more extensive which is in agreement with the results of Cleland et al. (1973).

One of the most conspicuous differences between the cat and Tupaia is the receptive field organization in the dark.

Mean or range	Stand. dev.	No.	Type	Animal	Exp. condition	Authors
0.0-5°			T+S	ground squirrel	fibres: retina	Michael (1968a)
2.0°	0.84	38	T+S	Tupaia	fibres: retina	this investigation
2.4°	0.55	21	T	Tupaia	fibres: retina	this investigation
1.3°	0.65	17	S	Tupaia	fibres: retina	this investigation
0.2-0.4°			S	cat	cells: area centralis (5°)	Ikeda & Wright (1972)
0.9°	0.24	31	S	cat	cells: area centralis (30°)	Cleland et al. (1973)
1.5°	0.65	21	S	cat	fibres: retina	Enroth-Cugell & Robson (1966)

Table 3.5 Mean diameter and standard deviation of the uniform center of sustained (S) and transient (T) units.

There are several indications that the surround mechanism is absent in the dark adapted state in the cat, but not in Tupaia (Table 3.6). This is probably responsible for the fact that the distinction between the two types of units in the cat has become apparent only in the last few years (Hamasaki and Winters, 1974). In the next chapter it will be shown that the differences between the two types are expressed in several other characteristics in the light adapted state.

	cat	*Tupaia*
surround mechanism	weak or absent (Barlow and Levick, 1969a; Cleland et al., 1973)	present (Fig. 3.19)
response type	sustained (Enroth-Cugell and Shapley, 1973)	sustained or transient (3.3.2.1)
inhibition	absent (Yoon, 1972)	present (Fig. 3.19)
QSR	2-14 (Barlow et al., 1971)	10^3 - 10^7 (Fig. 3.18)

Table 3.6 Differences in the dark adapted state between the cat and *Tupaia*.

From Fig. 3.5 and from similar data from other units it seem to us that the stimulus response curves do not intersect the ordinate at the level of the maintained activity but at a lower level. A small but probably significant threshold seems to be present. In Fig. 3.20 three plots of stimulus response curves are shown. Each plot is based on a particular assumption concerning a threshold in the element which transmits the signal through the retina. When there is no threshold at all the lines should be the ones shown in plot A. When each receptor has a certain threshold then the lines in plot B are to be expected, since the threshold number of quanta is then proportional to the number of receptors within the stimulus area. Neither group of response curves was ever observed. Plot C, which was observed in our units, is obtained with the assumption of a threshold in the bipolar cell (if one bipolar cell pools the output of the cones in the uniform center) or in the ganglion cell when several bipolars are pooled at a single ganglion cell. So it can be concluded from these plots that no threshold is present in the cones.

It has been argued that the wide range of QSR (Fig. 3.8) in the dark adapted state cannot be ascribed to the general condition of the animal or the transparency of the optical media. However, colour sensitivity may play an important role. Blue sensitive units, if they are present, would yield a high

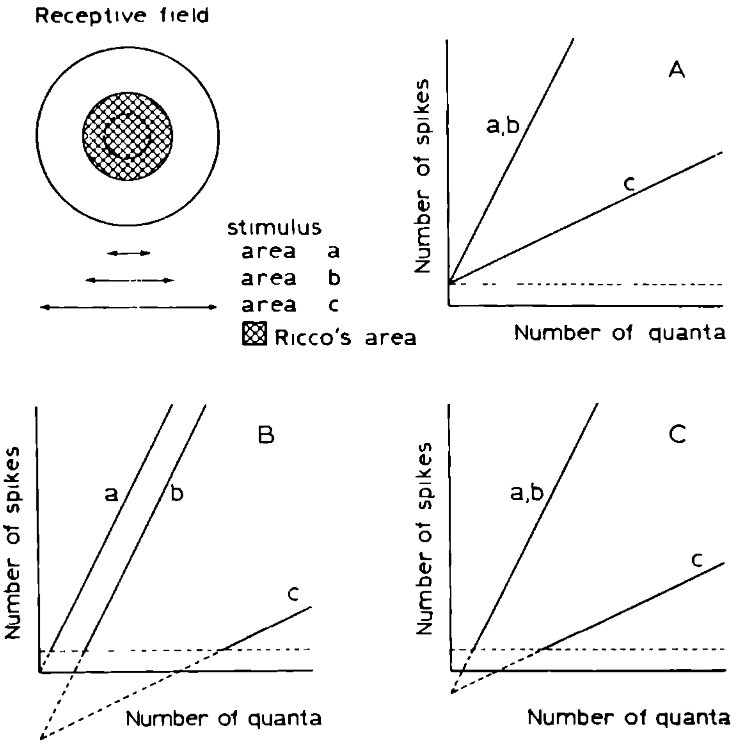

Figure 3.20 Stimulus response curves when stimuli with increasing diameters are presented and when thresholds in various cells of the retina are postulated. (see Discussion and Fig. 3.5).

QSR, because the green stimulus is a fairly ineffective one. Opponent colour units were found by Michael (1968c) in an all-cone retina. It is, however, improbable that there are many opponent colour units present in the sample of Tupaia for two reasons: 1^{o} The searching stimulus was a white one: opponent colour units are very insensitive to a white stimulus (Michael, 1968c). 2^{o} The spectral sensitivities of several units were measured; in these units the whole spectrum delivered only on or off responses for on-center and off-center units respectively. Only one opponent colour unit was found (see Appendix). Non-opponent blue sensitive units were not found.

In this investigation sustained and transient units were distinguished in an all-cone mammal. Both center mechanism and surround mechanism can be activated by either rods or cones or combinations of them since this classification can also be made in the cat with its rod dominated retina.

A functional correlate of the classification can be given. Sustained units are especially suitable for obtaining information about spatial contrast. Transient units are able to detect fast movements (Cleland et al., 1971) and possibly play an important role in triggering fixation reflexes and orientation responses, by which the eye or the head is so directed that the *area centralis* can obtain maximum information about spatial contrast (Ikeda and Wright, 1972). An additional argument is the fact that we have not found transient units in the ipsilateral eye, but only sustained units, which were present in the immediate vicinity of the *area centralis* (Fig. 3.16). The dorsal lateral geniculate nucleus probably receives a double input of sustained units via the two nasal retinas from one half of the binocular visual field, if it is supposed that all sustained units go to the geniculate body as in the cat. This is in accordance with the finding that in *Tupaia* each *striate cortex* (area 17) receives a double input via the two nasal retinas from one half binocular visual field (Hall et al., 1972). The *superior colliculus* of *Tupaia*, to which only transient units project, receives inputs from the contralateral eye only (Lane et al., 1971) and this is in accordance with our observation that no ipsilateral transient units were present.

It is probable that in the *area centralis* the smallest receptive fields are present and this would be in agreement with behavioral experiments (Ordy and Samorajski, 1968), which have shown that the acuity of *Tupaia glis* is about 1 - 2 (1' - 0.5').

3.5. Summary

1. Single unit recordings of on-center units were made from optic tract fibres of the all-cone retina of *Tupaia*, a primitive monkey.
2. Uniform center size was measured by plotting the Quantum to Spike Ratios (QSRs) as a function of stimulus diameter. Ricco's area was taken as the criterium for the uniform center size. The retina was dark adapted.
3. With the aid of long square wave pulses presented to the uniform centers, the whole population of on-center units could be divided into two groups. In one group the steady

state activity was increased during light-on: sustained units. In the other group this activity was about the same as the maintained activity in the dark: transient units.

4. The burst duration as a result of long or short rectangular pulses was longer in sustained units than in transient units.
5. Sustained units had smaller uniform centers than transient units. The border of the uniform center of sustained units was more distinct than that of transient units.
6. In the uniform center of sustained units no suppression during light-on was observable. In the uniform center of transient units the surround mechanism was observable by the suppression following the burst at the onset of light and by the smaller burst after the suppression at the offset of light. This was confirmed by the low increase in the steady state activity during light-on and the low decrease in activity during light-off.

   It is concluded that both during dark adaptation and during light adaptation the surround mechanism in the uniform center is weak or absent in sustained units and strong in transient units.
7. Diffuse stimulation of sustained units revealed responses which were similar to the center responses of transient units. Diffuse and center stimulation of transient units produced the same responses.

   It is concluded that in the receptive field periphery the center mechanism is weak or absent in sustained units and is strong in transient units.
8. Sustained units are concentrated in the region of the *area centralis*. All ipsilateral units were sustained units and located in the binocular visual field.
9. The QSRs of the units in the dark adapted state ranged from 10^3 to 10^7.
10. No correlation existed between the type of units and the maintained activity in the dark.
11. In the light adapted state the differences between the two types with respect to the burst duration diminished. The steady state activity, however, remained visible in sustained units.
12. This study shows that the two types of units which are found in the cat's retina, also exist in an all-cone retina, and moreover the classification holds true also during dark adaptation. Hence the classification holds for both rod and cone systems. It demonstrates the functional significance

of each of the types independent of a diurnal or nocturnal mode of life.

3.6. Appendix: other types of units

As well as on-center units (Fig. 3.21A and C) other types of units were also found. These units were not as extensively studied as the on-center units. For the sake of completeness all units found will be considered and compared with literature in this section.

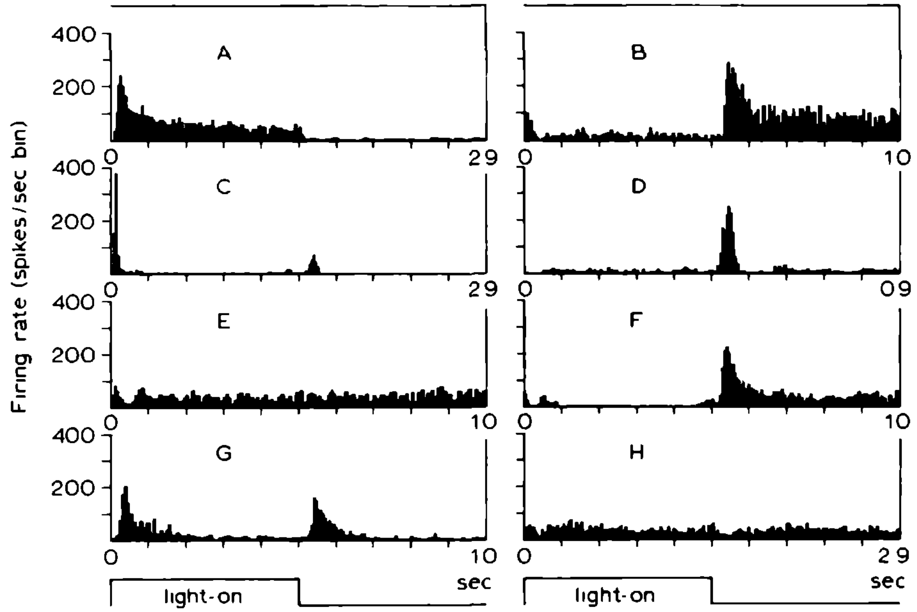

Figure 3.21 Post Stimulus Time Histograms of different types of units. A: sustained on-center unit; B: sustained off-center unit; C: transient on-center unit; D: transient off-center unit; E, F: edge inhibitory off-center unit responding to a small (0.25°) stimulus and a large (1°) stimulus respectively; G: on-off unit; H: suppressed by contrast unit.

Off-center units and on-center units were found in about equal numbers. The center area of off-center units was somewhat larger than that of on-center units. As in the case of on-center units the off-center units can be divided into two types; sustained and transient units (Fig. 3.21B and D).

On-off units (7) responding to both the onset and offset of light with about the same latencies were also found (Fig. 3.21G). The 'local edge detector', 'on-off cells' and 'excited by contrast' cells of the cat (Barlow et al., 1964; Cleland and Levick, 1974; Levick, 1967; Stone and Fabian, 1966; Stone and Fukuda, 1974; Stone and Hoffmann, 1972) are probably units of the same types as the on-off units in Tupaia.

Two units with a relatively high maintained activity (15 spikes/sec) were found, which activity was suppressed by the onset and the offset of light (Fig. 3.21H). Units with these characteristics, termed suppressed by contrast units or 'uniformity detectors', have also been found in the rabbit (Levick, 1967) and in the cat (Rodieck, 1967; Stone and Fukuda, 1974; Stone and Hoffmann, 1972).

Directional selective units (16) were often found in Tupaia. These units responded maximally to a stimulus (dark or light) moving in the preferred direction. Movement of the stimulus in the opposite direction suppressed the maintained activity totally. These units have also been described in the ground squirrel (Michael, 1968), the rabbit (Barlow et al., 1964; Levick, 1967) and in the cat (Cleland and Levick, 1974; Stone and Fukuda, 1974; Stone and Hoffmann, 1972).

All the above described units responded more strongly to small stimuli than to extensive stimuli. So all these units appear to have an inhibitory surround mechanism.

In some units (6) it was possible to elicite both sustained on- and sustained off-responses by stimulating with small spots in different parts of the receptive field in the dark. These units had elongated receptive fields. Diffuse stimulation yielded vague and variable responses. It is possible that these units belong to the same type, viz. orientational selective units, as described for the rabbit (Barlow et al., 1964; Levick, 1967). In the cat elongated receptive fields have also been described (Cleland and Enroth-Cugell, 1968).

One opponent colour cell was found. The center was sensitive to both blue (440 nm; on-response) and red (560 nm; off-response). Stimuli at about 500 nm yielded about equally strong on- and off-responses. The peripheral part of the receptive field was sensitive to red only. It was possible to elicit on-responses from the red sensitive surround mechanism by adapting the center (with red). Behavioural experiments with Tupaia (Polson, 1968) indicate that the retina is sensitive to both blue and red.

One unit was found which showed a short suppression only at light-on with small centered stimuli; larger stimuli yielded off-responses (Fig. 3.21E and F). These response characteristics have also been found by Cleland and Levick (1974), however, with moving stimuli. They called these units edge inhibitory off-center cells.

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CHAPTER 4

INFLUENCE OF ADAPTING SPOTS ON THE RESPONSES OF ON-CENTER UNITS IN THE ALL-CONE RETINA OF TUPAIA CHINENSIS

4.1. Introduction

In the work described in the previous chapter two types of on-center units were found, viz. sustained units and transient units. A model, based on these results, was presented. In this chapter the properties of both types of units with respect to light adaptation will be considered. The influence of diffuse illumination and of illumination of the uniform center only will be described. It will be shown that the maintained activity as a function of light adaptation is different for the two types of units.

Enroth-Cugell and Shapley (1973b) found an inverse proportionality between the uniform center area and the level of background illumination at which light adaptation starts. The same relationship has been investigated in this study and it will be shown that different responses are found in the two types of units.

The adaptation pool also differs for the two types and the results explain the discordance found in the literature (Enroth-Cugell and Shapley, 1973b and Sakmann et al., 1969 on the one hand and Maffei et al., 1970 on the other hand).

With adapting spots in the center the response of both types of units to flashing annuli was studied. The results support the idea of a different organization in the two types of the two mechanisms (viz. the center mechanism and the surround mechanism) described in chapter 3.

Furthermore, latency differences between the two types were studied. Stone and Hoffman (1972) found in the cat different conducting times for the two types.
Transient units conduct spikes faster than sustained units.

Lastly the influence of background illumination on the latency will be described.

4.2. Methods

The experiments discussed in this chapter have been carried out using the same methods as described in chapter 3. The only addition concerns the use of annular stimuli.

In this chapter Increment threshold curves as a function of background illumination will be presented. The Quantum to Spike Ratio (QSR) is used as the Increment threshold, rather than a stimulus magnitude (ΔI) referring to one value of a response measure. This does not matter since there is a linear relationship between the number of extra spikes and ΔI, and because the stimulus response curves intersect the ordinate at about zero 'extra' spikes. So the shape of the Increment threshold curves is identical for different magnitudes of the response criterium, provided that these magnitudes (extra spike numbers) are chosen in the linear range of the stimulus response curves. An extensive treatment of the Increment threshold curves with different criteria will be presented in the next chapter.

4.3. Results

4.3.1. Increment threshold curves as a function of background illumination

Stimulus response curves were measured with the background illumination as variable. Background illumination was increased in one log unit steps. Fig. 4.1 shows such stimulus response curves for one transient unit. The response was measured as the number of spikes in the first 100 msec after onset of the stimulus. It can be seen that the lower part of these curves approximates to a straight line. The slope of these curves gives the inverse QSR. The maintained activity at each background level is indicated at the left side of the ordinate. In Fig. 4.2 the QSR of the stimulus response curves for two units has been plotted against the background illumination used. The QSR and the background illumination are expressed in relative units because of differences in the dark QSRs and in the intensities at which light adaptation began. The curves were shifted along the abscissa so that the points on the curves, where the QSR was increased half a log unit, coincided. The dark QSR for all units has been adjusted to 1.

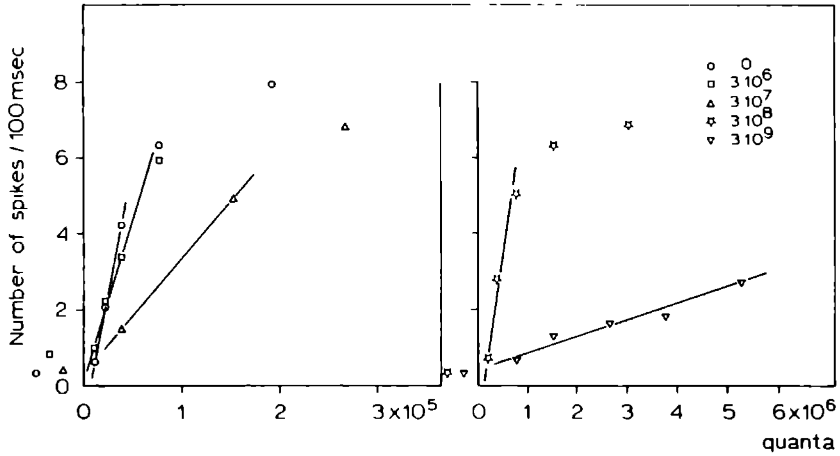

Figure 4.1 Stimulus response curves in the dark and light adapted state. The maintained activity is indicated at the left of the ordinate. Pulse duration 10 msec. Stimulus diameter inside the uniform center. Unit 35-2 (transient).

It can be seen that the shapes of both curves are very similar. The broken line in this figure indicates the operation of Weber's law, viz. the proportionality between QSR and background illumination. This relationship is reached, on the average, at the two highest background intensities for the two units. The background illumination at the intersection of the line with unity slope and the dark QSR (the ordinate) is in general somewhat smaller (Fig. 4.3) than the background illumination at half a log unit sensitivity decrease (I_t). The last mentioned background illumination (I_t) will be used as a measure for the beginning of light adaptation.

Curves have been measured for 8 sustained and 17 transient units. After standardising the curves as described above there is still some variability, but there is no difference in shape when sustained units are compared with transient units (Fig. 4.3A and 4.3B). In psychophysics the slope of the curves is dependent on the area and the duration of the stimulus (Barlow, 1957; Bouman, 1952). In our experiments there is no difference in the shape of the curves whether the uniform center or a much more extensive region (up to six times the uniform center area) was stimulated. This was done for six units (Fig. 4.3C).

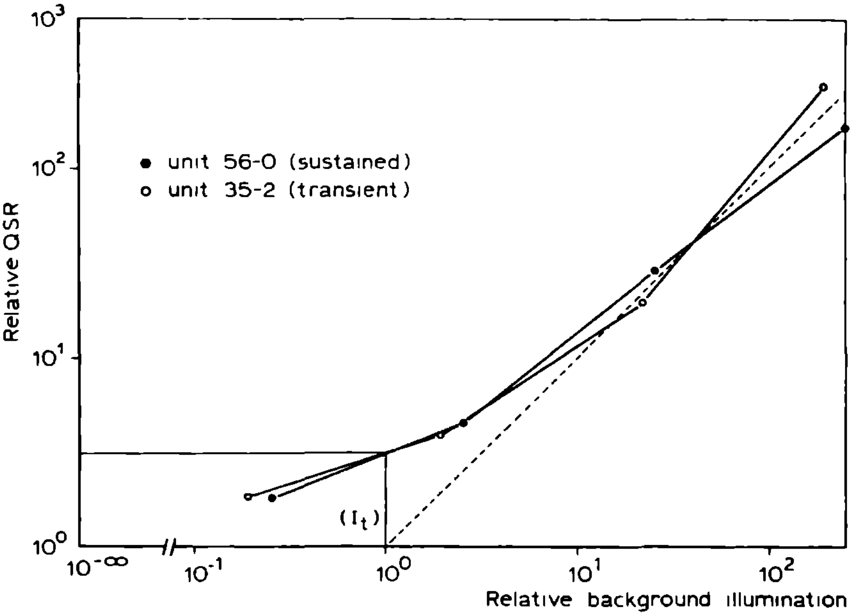

Figure 4.2 Increment threshold curves as a function of background illumination. The curves are shifted along the ordinate so that both dark-QSRs coincide (relative QSR = 1) and are shifted along the abscissa so that both points on each curve at half a log unit above the dark-QSR coincide. The background illumination belonging to these points will be called I_t and is set at 1. Stimulation inside the uniform center. Pulse duration: 10 msec. The broken line indicates Weber's law.

4.3.2. Burst duration and maintained activity as a function of background illumination

From Table 4.1 it can be seen that an increase in the background illumination caused a shortening of the burst duration. This increase also caused a raised maintained activity in the sustained unit (56-0) but did not significantly change the activity of the transient unit (35-2).

It was a general observation that an increased QSR generated by background illumination is coupled with a shortening of the burst duration in both types and with a raised maintained activity in sustained units.

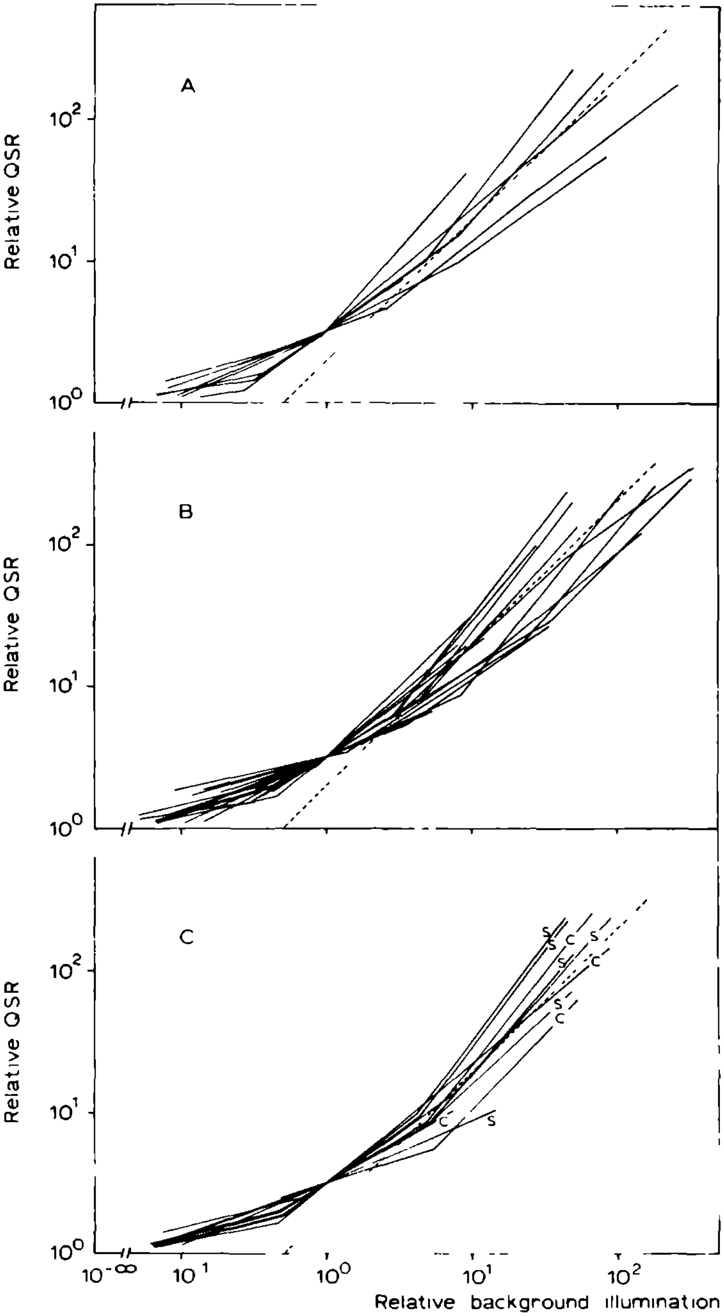

Figure 4.3 Increment threshold curves as a function of background illumination. A: sustained units; B: transient units; C: stimulation inside the uniform center (c) or in a more extensive region (s). A, B and C: pulse duration: 10 msec. The broken lines indicate Weber's law.

Relative background	Maint. act.		Burst duration	
illumination	56-0	35-2	56-0	35-2
dark	1.5	0.35	130	65
2.10^{-1}	1.7	0.8	100	40
2.10^{0}	3.0	0.4	45	30
2.10^{1}	3.8	0.35	30	20
2.10^{2}	3.8	0.35	20	20

Table 4.1 Example of the influence of background illumination on the maintained activity (number of spikes/ /100 msec) and the burst duration (msec; criterium at 4 extra spikes) for a sustained (56-0) and a transient unit (35-2).

Because the sensitivity for light adaptation is different from unit to unit and because the burst duration and the maintained activity only change, when the 'dark-QSR' has changed due to light adaptation, these two quantities will be considered in connection with the QSR.

Concerning the burst duration, it was already known from the results described in the previous chapter (Fig. 3.10 and Fig. 3.12) that the burst duration becomes shorter with light adaptation for both types of units and that the distinction in this respect between the two types diminishes with light adaptation. In Fig. 4.4 four histograms are presented; two observations can be made: 1° the burst duration of sustained and transient units becomes shorter with light adaptation, 2° this decrease is much more pronounced in sustained units, so that at the highest level no difference between the two types is observable.

The decrease in the burst duration was also found in the cat by Enroth-Cugell and Shapley (1973a) and they ascribed this to a shortening of the time constant of the center mechanism with adaptational level.

The maintained activity in Tupaia was measured in the dark and at different background illuminations for the two types of units. It appeared (Fig. 4.5A) that the maintained

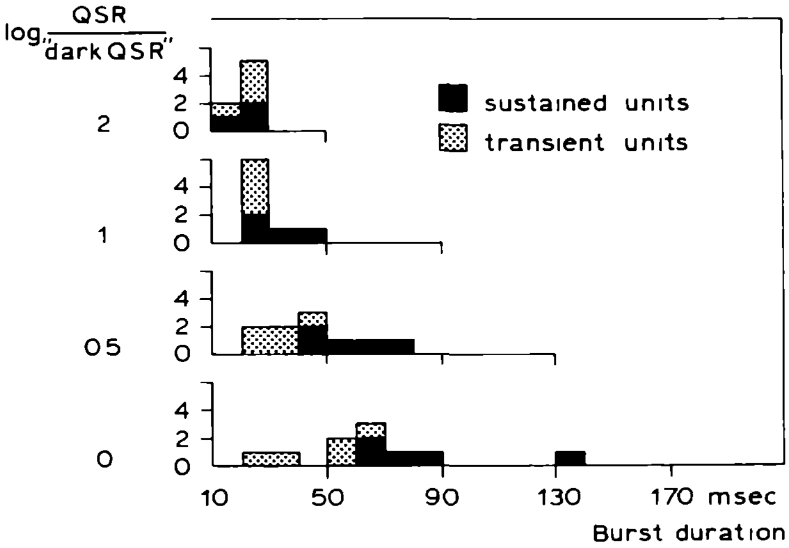

Figure 4.4 Histograms of the burst duration with the level of light adaptation (expressed in log units above the dark-QSR at the left of the ordinate) as the parameter. Stimulation inside the uniform center. Stimulus duration: 10 msec.

activity of transient units do not show, on the average, any change with background illumination, while this activity of sustained units increases with light adaptation and levels off at background illuminations, which cause a QSR-increase of one and a half log unit.

If also the maintained activity is the result of the contributions of a center and a surround mechanism it means that both mechanisms in transient units are very well balanced for all background illuminations used. In sustained units the maintained activity increases as a function of light adaptation, so the surround mechanism is weaker with respect to the center mechanism for all background illuminations used than in transient units.

Evidence that the maintained activity is pooled in the center in sustained units is apparent in Fig. 4.6. A steady spot was placed in the center (uniform center size is indicated by arrows) and the maintained activity was measured. The spot size was increased while holding the flux constant. So the increase in the spot area by a factor of four was coupled

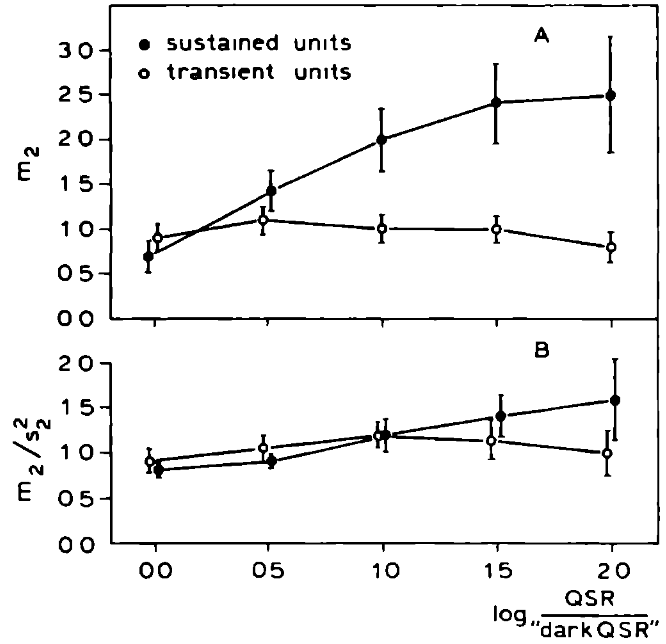

Figure 4.5 A: maintained activity (m_2: mean number of spikes in 100 msec) and B: regularity (m_2/s_2^2; s_2^2 is the variance of the number of spikes) for sustained (10) and transient units (15) as a function of the level of light adaptation. The standard deviation of the mean for each point is indicated.

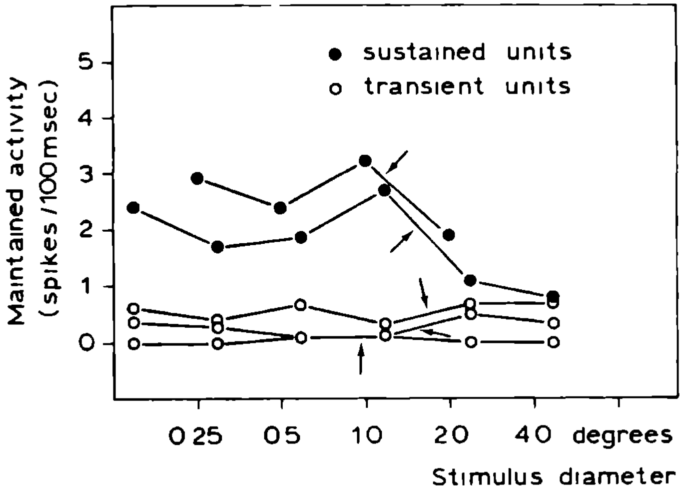

Figure 4.6 Maintained activity as a function of steady illumination with increasing disc areas. Arrows indicate the uniform center size.

with a decreased illumination of the same magnitude. In both types of units the maintained activity stayed roughly unchanged in the uniform center, but as soon as the spot also covered a part of the periphery, maintained activity decreased in sustained units and did not change in transient units.

When increasing discs of the same illumination were used in sustained units, the maintained activity increased, decreased and increased again in most cases, revealing, respectively, the uniform center, the receptive field periphery and a region probably similar to the so-called 'disinhibitory surround' (Ikeda and Wright, 1972a).

In Fig. 4.5B the ratio of mean and variance of the number of spikes over 100 msec during the steady state has been plotted as a function of QSR. This measure of regularity stays approximately unchanged in transient units and increases slowly in sustained units. The increase in maintained discharge is coupled with an increase in the variance of the number of spikes in sustained units, while in transient units the variance remains the same or even diminishes. So the variances are related to the mean, but in a complex fashion.

4.3.3. Relation between center area and light adaptation

As an indication for the beginning of light adaptation the background illumination at 0.5 log unit above the dark QSR of each unit was taken (I_t). Although there was some scatter it appears that there is an inverse proportional relationship between the uniform center area and I_t for transient units (Fig. 4.7). This dependency is absent in sustained units.

It is probably that the adaptation pool of the transient units is as extensive as the center because:

1° the pool coincides with the center in the cat (Enroth-Cugell and Shapley, 1973b); see also next section.

2° there exists an inverse proportional relationship between the uniform center area and I_t (Fig. 4.7).

However, this relationship does not hold for the sustained units in *Tupaia* and the possibility exists that the periphery, as well as the center, also plays an important role and may even be responsible for the absence of this relationship.

So the variation in I_t cannot be explained by the variation in center area. The possibility exists, that the

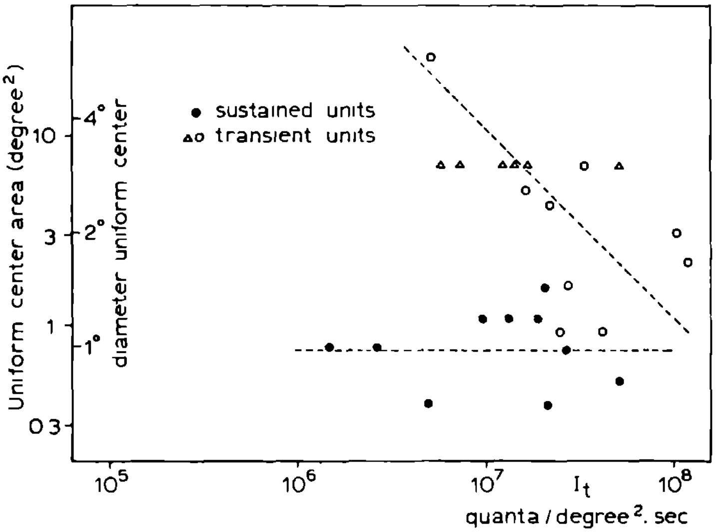

Figure 4.7 Uniform center area as a function of I_t (background illumination at a defined low level of light adaptation; see also Fig. 4.2) for sustained and transient units. Triangles: uniform center diameter between 2^0 and 4^0. Correlation coefficient of the transient units: -0.7 and of the sustained units: 0.1.

variation in I_t is due to the variation of the strength in the surround as compared with the strength of center. The hypothesis is suggested that a high value of I_t is due to a relatively strong surround and vice-versa.

We have already correlated the level of maintained activity with the strength of the surround. Illuminating the periphery decreases the maintained activity, which shows that the exitation of the surround decreases the maintained activity significantly. The maintained activity increases on the average with the adaptation level as can be seen from Fig. 4.5. In this model we assume that the greater the strength of the surround the smaller would be the increase in the maintained activity with adaptation level. Therefore the slope-value of the curve which connects the maintained activity with the level of light adaptation was measured for each unit (in Fig. 4.5A the mean slope of all units is plotted). This value is plotted against the I_t-value for each unit (Fig. 4.8). It can be seen from this figure that the slope-values are

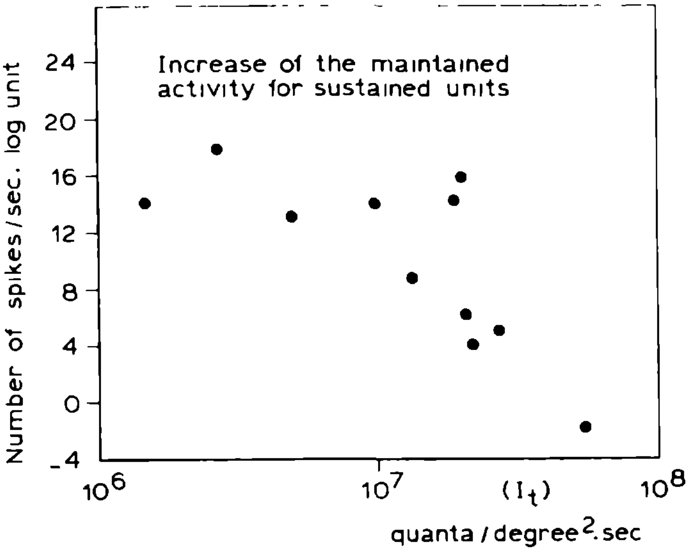

Figure 4.8 Slope values of the maintained activity as a function of I_t for different sustained units. The mean slope values of the maintained activity as a function of light adaptation (ordinate) can be calculated from Fig. 4.5A. In that figure the slope is indicative for the mean increase of all sustained units.

negatively correlated with the I_t-values. This is in agreement with the assumption made above that units with a relatively weak surround have relatively low I_t-values.

This also means that the adaptation pool does not coincide with the center, as is the case in transient units, but extends further peripherally about the center (see next section).

The maintained activity in the dark of sustained units with a low I_t or a weak surround is higher than in units with a strong surround or high I_t. Indeed there exists a strong negative correlation (Fig. 4.9). Transient units on the other hand are more active in the dark the higher the I_t-values. Although there is some scatter, this correlation being positive, is clearly less than in sustained units. But a correlation of I_t with the strength of the surround mechanism (measured in slope-values of the maintained activity) is not evident. The absence of a clear correlation between slope-values and I_t-values can be understood because the location on the I_t-axis is primarily a matter of uniform center size

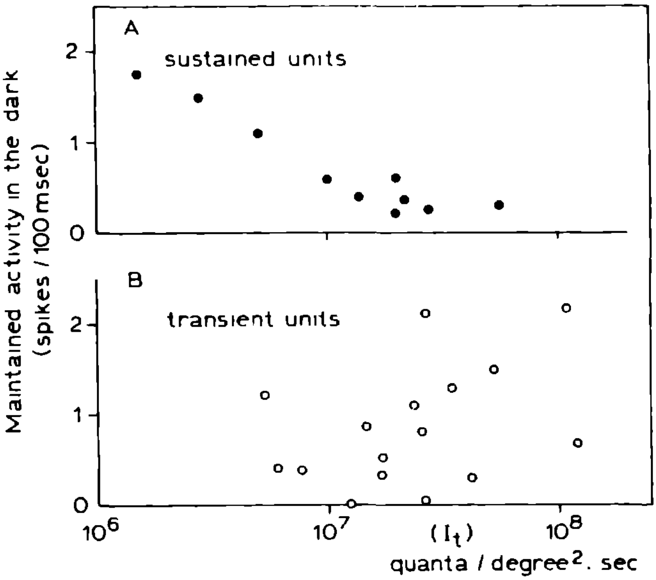

Figure 4.9 Maintained activity in the dark as a function of I_t for (A) sustained and (B) transient units. Correlation coefficient for the sustained units: -0.9 and for the transient units: 0.3.

in transient units. In sustained units it is mainly a matter of the strength of the surround mechanism, because these units are rather homogeneous with respect to the center size in our sample.

4.3.4. The adaptation pool in sustained and transient units

The adaptation pool is defined here as that area of the receptive field in which continuous stimulation changes the QSR (measured with short pulses) of the uniform center.

The adaptation pool was measured by using increasing adapting fields (0.6 - 15°) of the same illumination. At each adapting field a stimulus response curve was measured by using short flashes presented in the uniform center. The QSR was measured and plotted against the adapting area (Fig. 4.10, broken lines). The uniform center size was also measured using increasing stimulus diameters in the dark adapted state (Fig. 4.10, solid lines). In both the sustained and transient unit the QSR increases in the uniform center (about proportional with the adapted area), levels off and then does not change in the transient units, but decreases in the sustained units. The same characteristics were found in two other units. These results indicate that both center and surround mechanism are involved in the adaptation pool. In the transient units no region with an inhibitory influence on the

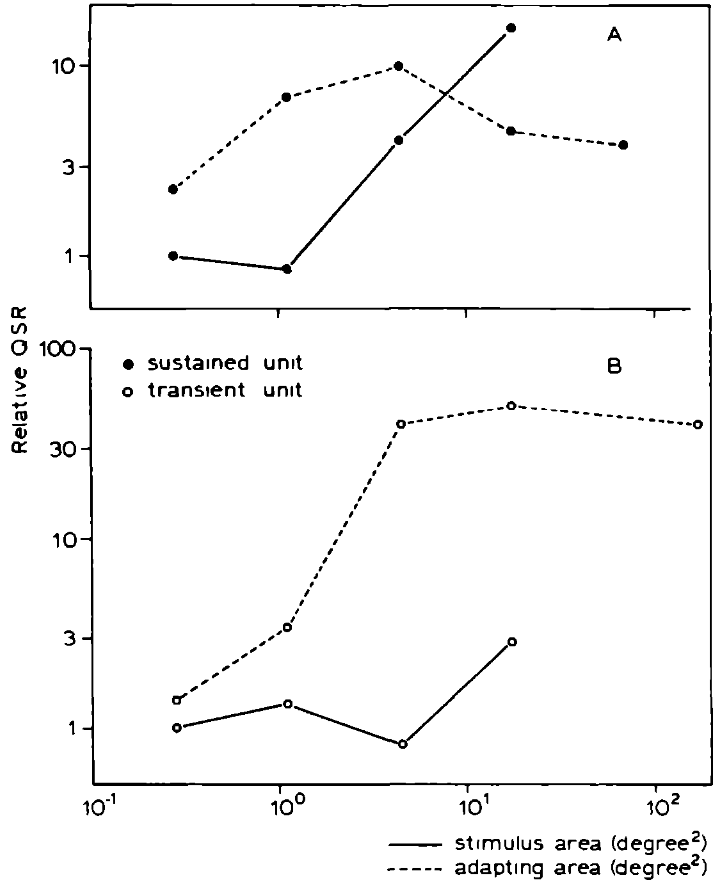

Figure 4.10 Relative QSR (the dark-QSR is set to 1) as a function of the adapting area (constant illumination) for (A) sustained and (B) transient units (broken lines); stimulus (0.6°) inside the uniform center; stimulus duration: 10 msec.
The uniform center size can be measured from the stimulus area-QSR curves in the dark (solid lines).

central part of the adaptation pool could be established, whereas this region was demonstrable in the sustained units. So it is probable that the adaptation pool in both types consists of the same elements but with a different spatial arrangement just as described for the superposition models in chapter 3.

4.3.5. Influence of adapting spots in the center on the responses to annular stimuli

In the preceding sections it was shown that adaptation by diffuse illumination diminishes the response on short pulses. It is also clear from the preceding chapter that in the uniform center the surround mechanism is weak or absent in sustained units and is strong in transient units. There are indications (section 4.3.4) that in transient units the adaptation pool coincides with the center. The presence of the two mechanisms in the center suggests that both center and surround mechanisms are involved in forming the pool. In sustained units, however, the center and surround mechanism are spatially better separated than in transient units and in these units adaptation of the center would be more effective than in transient units in eliciting responses from the surround mechanism.

In this section the responses to stimulation of the receptive field periphery with varying center adaptation will be described. About 15 sustained and transient units were investigated. One representative sustained and one representative transient unit will be considered. In the upper part of Fig. 4.11 two dot-displays of one sustained unit are shown. In the left one responses to long square wave pulses of light in the periphery are shown and in the right one responses to short pulses. The illuminations of the adapting spot in the center increases stepwise from top to bottom. It can be seen that stimulation of the periphery without illuminating the center elicits an activation at light on. The burst duration is short and the level of steady state activity is hardly higher than the maintained activity in the dark. So the response has the same characteristics as the center response of transient units (chapter 3). Illuminating the center with a low intensity does not change the response up to a value of 10^6 quanta/degree^2.sec. At 10^7 quanta/degree^2.sec the response changes. An activation during

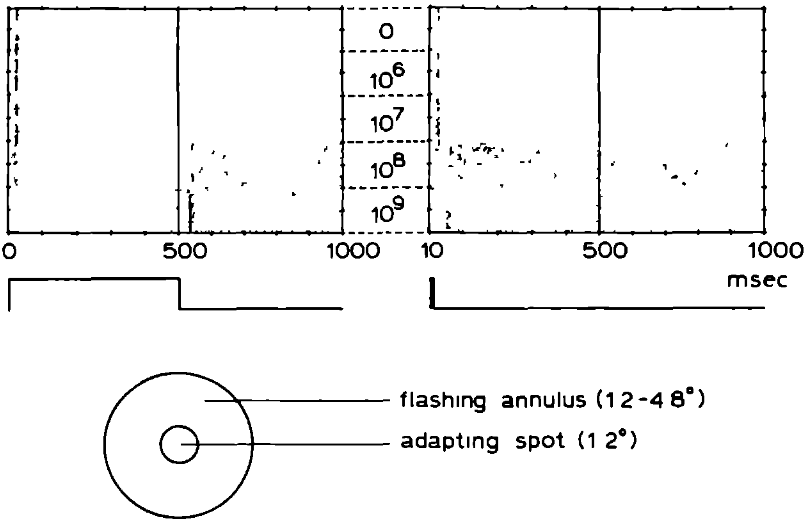

Figure 4.11 Two dot-displays illustrating the influence of an adapting spot on the responses to a flashing annulus of constant intensity. The adapting spot and the uniform center coincide. Left figure: long square wave stimulation; right figure: short pulse (10 msec) stimulation; adapting illumination in quanta/degree^2.sec. The inner diameter of the adapting spot and both the inner and the outer diameter of the annulus are indicated. Unit 49-3 (sustained).

light-off becomes visible and at the highest illumination level the activation during light-on has disappeared. The response shape resembles the center responses of sustained units, but it is a sustained-off response. So the surround mechanism is dominating. The latencies of the responses at light-on and at light-off differ (15 and 29 msec respectively) indicating that the mechanisms have different latencies. The response on short pulses shows the same characteristics. The burst following short pulses decreases with light adaptation of the center. Another burst with a longer latency resulting from the surround mechanism appears at a high level of light adaptation.

Fig. 4.12 shows the responses of a transient unit for which the same procedure was followed. Instead of dot-displays PSTHs are now presented. Also in this unit low levels

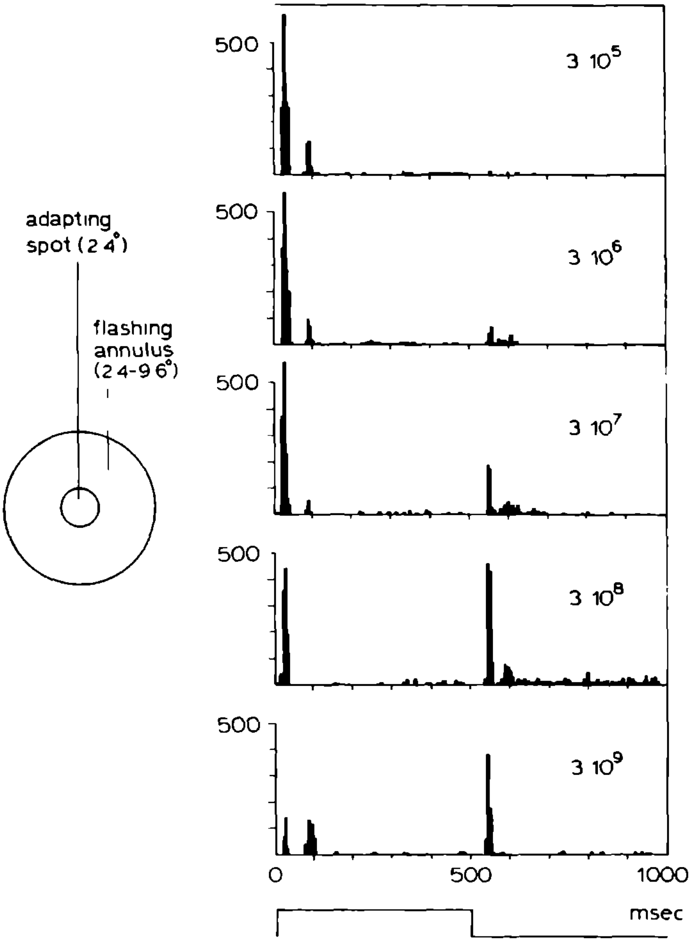

Figure 4.12 PSTHs illustrating the influence of an adapting spot on the response to a long flashing annulus of constant intensity. The adapting spot and the uniform center coincide. The inner diameter of the adapting spot and both the inner and the outer diameter of the annulus are indicated. Unit 50-0 (transient).

of center adaptation do not change the response. The response from the periphery without illuminating the center, resembles a center response of this unit. A second burst in the light-on phase can be seen, which is possibly caused by the shorter time constant and the longer latency of the surround mechanism. Illuminating the center with intenser spots yields weaker responses during light-on and stronger responses during light-off up to the penultimate intensity used. In the light-off phase a second burst is also present. The last used intensity, however, decreased the response at the offset of light and both at light-on and light-off responses were present. So it is probable that both mechanisms are involved. The latency of the first burst at the onset of light is 20 msec and that of the first burst at the offset of light 35 msec. So the surround mechanism has a longer latency than the center mechanism even at this high level of center adaptation.

Adaptation by diffuse illumination with stimulation (10 msec pulses) of the uniform center or of a more extensive area never yielded an off-burst with a long latency (as shown in Fig. 4.11 in the lower half of the right hand figure), which would be caused by the surround mechanism. This means that not only the center mechanism is adapted, because in that case responses from the surround mechanism with a long latency would be expected. So both center and surround mechanisms are adapted by diffuse illumination which is in accordance with the results discussed in the previous section.

From the above mentioned results in this section there are no indications that the spatial configuration as found in the sustained units is also present in the transient units.

4.3.6. Latencies of sustained and transient units stimulated with short pulses as a function of different background illuminations

If the well-known differences in the conducting velocities (determined by measuring the antidromic latencies in the optic disc after electrical stimulation by electrodes placed in the optic chiasm or optic tract) in fibres of sustained and transient units in the cat also apply to *Tupaia*, a difference of 0.2 msec between the latencies of both types should be found.

If for the *Tupaia* in the dark adapted state the latencies were plotted as a function of stimulus energy using 10 msec pulses restricted to the uniform center, then, for each unit one latency curve was obtained. If latency was plotted against stimulus intensity using varying stimulus areas inside the uniform center, then several curves were obtained for each unit. Stimuli which exceed the uniform center become more and more ineffective, because quanta falling outside the center do not contribute to the extra spikes. So the stimulus energy has to be corrected for stimuli which exceed the center. This can be done by dividing the energy by the same factor with which the QSR at that stimulus diameter has been increased with respect to the QSR of the uniform center. When this is done all these latency curves coincide (Fig. 4.13A and B). Because in the dark the latency of a burst caused by a stimulus in the center is a function of stimulus energy and because stimulus energy present in short pulses is directly related to the number of extra spikes, the latency can also be plotted against the number of extra spikes elicited by short pulses. This has been done for 5 transient and 6 sustained units (Fig. 4.14A). It can be seen that there is great variability of latencies between units. This variability is mainly caused by the fact that separate units show longer

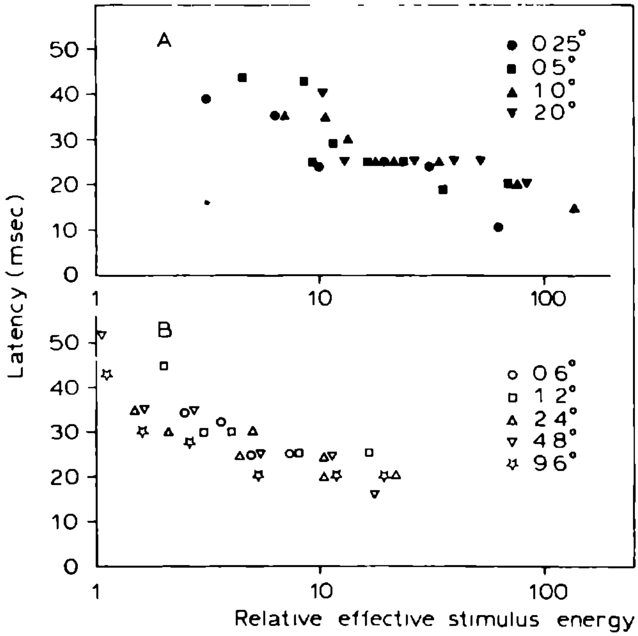

Figure 4.13 Latency as a function of the relative effective stimulus energy for a sustained (A; 44-0) and a transient (B; 50-0) unit in the dark adapted state. The stimulus diameters are indicated. Stimulus duration: 10 msec.

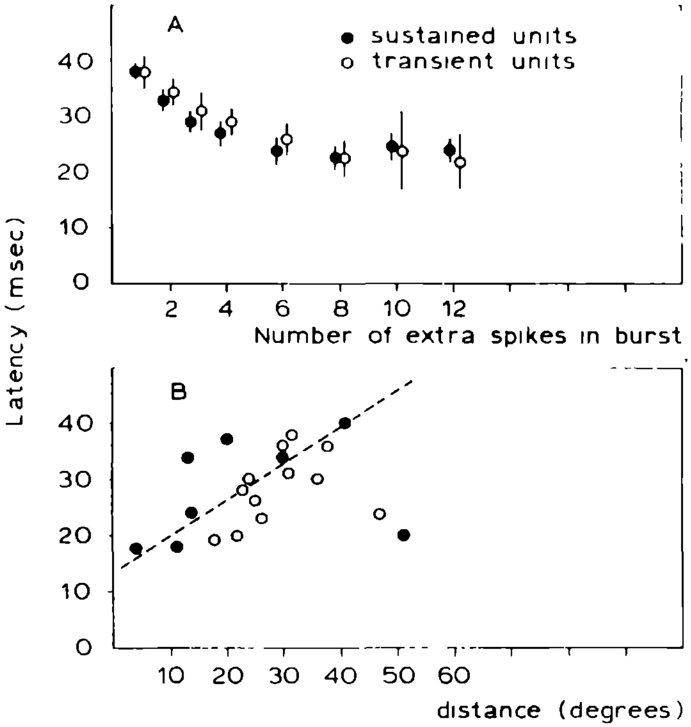

Figure 4.14 A: Latencies in the dark adapted state for both sustained (6) and transient (5) units at short (10 msec) pulses as a function of the response magnitude. B: latencies in the dark adapted state as a function of the distance from the optic disc. A part of the variability among the units (Fig. A) can be explained by the different distances of the fields from the optic disc, since there is a positive correlation in Fig. B. The standard deviation of the mean is indicated in A. Latencies in B were calculated at a criterium of 4 spikes in the burst. Stimulation inside the uniform center with short (10 msec) pulses.

or shorter latencies over their whole dynamic range. It is possible that the difference in the lengths of the unmyelinated parts of the fibres (with slower conducting velocities) are responsible for these differences. The distances of the receptive fields from the optic disc should then be correlated with the latency. Fig. 4.14B shows this, thus explaining a great deal of the variability between units.

Differences of 0.2 msec are not discernable. There is no latency difference between the group of sustained and the group of transient units. Ikeda and Wright (1973) did not find latency differences between the two types provided that the stimulation was adjusted to the center size.

In Fig. 4.15A and B latencies have been plotted as a function of stimulus intensity (10 msec pulses) with the background illumination as a parameter. It can be seen that latency is a function of the stimulus intensity used and that a change in background illumination does not alter the latency significantly, although a wide range of response magnitudes (extra spike number in the burst) were present. In Fig. 4.16A each curve represents latencies of responses to the same

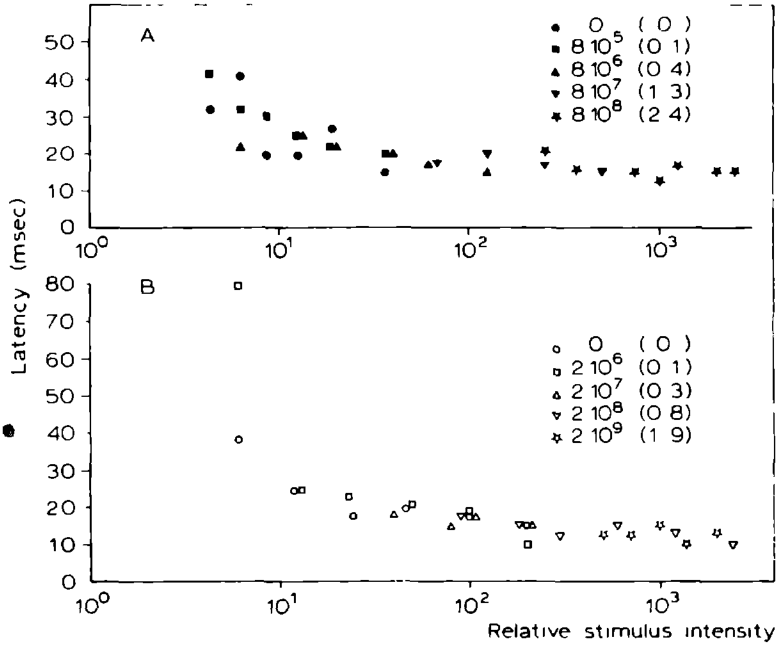

Figure 4.15 Latency as a function of the relative stimulus intensity with the level of background illumination as the parameter. A: sustained unit (46-0); B: transient (42-3) unit. The background illumination and the level of light adaptation (between brackets: log units above the dark-QSR) are indicated in this figure. Stimulation inside the uniform center. Stimulus duration: 10 msec.

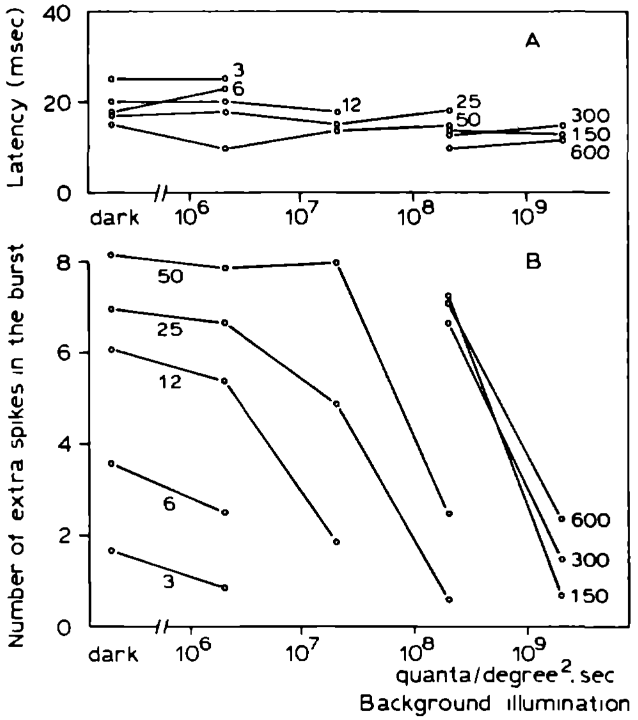

Figure 4.16 Latency (A) and response magnitude (B) as a function of background illumination with the stimulus intensity as the parameter. The connected points represent responses to stimuli with the same intensities (the relative intensities are indicated). Stimulus inside the uniform center. Stimulus duration: 10 msec. Unit 42-3 (transient).

stimulus intensity. These curves appear to be almost horizontal. Thus latency is independent of the level of background illumination but dependent on the stimulus intensity itself, which can also be concluded from the results shown in Fig. 4.15. In Fig. 4.16B each curve represents response magnitudes caused by the same stimulus as in Fig. 4.16A. The responses (number of extra spikes in 100 msec) are dependent on the background illumination. It can be concluded from Fig. 4.16A that the latency is only dependent on the stimulus intensity. From Fig. 4.16B it appears that the connection between the latency and the response magnitude depends on the background illumination. These conclusions apply to both sustained and transient units.

4.4. Discussion

In the previous chapter a model was proposed to explain the results from two types of on-center units. It is the same model as proposed by Hickey et al. (1973) who have used it to explain their results concerning on-center units in the cat. For both types two mechanisms, viz. a center mechanism and an inhibitory surround mechanism, are present. The surround mechanism has a longer latency than the center mechanism. In transient units both mechanisms overlap and in sustained units both mechanisms are spatially more separated.

The results from the adaptation pool experiments (Fig. 4.10) indicate that the adaptational flux inside the uniform center increases the QSR by the same factor in both types of units.

In the sustained units in the uniform center only a center mechanism is present, while in the transient units both a center and a surround mechanism are present. The conclusion must be that each of the mechanisms obey Weber's law because the results of the center mechanism only (sustained unit) as the result of both mechanisms together (transient unit) display this proportionality (Fig. 4.3).

That both mechanisms are indeed involved in light adaptation can be concluded from PSTHs used for Fig. 4.3. The responses to short pulses presented in the uniform center or in a more extensive area always have short latencies indicating that the responses are center-dominated. This means that the surround mechanism is also adapted, because if it were not adapted, at least the transient units would show responses with long latencies (off-burst as in Fig. 4.11 in the lower half of the right hand figure). So it can be concluded that both mechanisms are involved in light adaptation and that the center and surround mechanisms of both types behave in the same manner, viz. follow Weber's law at high background intensities.

The shortening of the burst duration (4.3.2) was also observed by Enroth-Cugell and Shapley (1973a) in the cat. They explain the shortening and also the shape of the Increment threshold curves, by a non-linear feedback model, in which the exponential function of the horizontal cell potential controls transmission from rods to bipolars.

The shortening of the burst duration and the diminishing number of extra spikes caused by long term light adaptation cannot be explained by the model in chapter 3. This is an *additive* model based on responses resulting from sudden changes in luminance at a certain adaptation level. At another adaptation level the same model will function but with other time courses and another gain. So the differences between the responses at different adaptation levels have to be explained by *multiplicative* factors such as those suggested by Enroth-Cugell and Shapley (1973a).

It was found that the maintained activity as a function of background illumination increases in sustained units and does not change, on the average, in transient units (4.3.2). Barlow and Levick (1969) and Sakmann and Creutzfeldt (1969) measured maintained activity in the cat. In both publications the maintained activity appeared to be dependent on the level of background illumination, when mean curves were presented. Yoon (1972) showed that the beginning of light adaptation is correlated with a change in maintained activity in the cat, just as in the case of the sustained units of *Tupaia*. Therefore we prefer to measure the maintained activity as a function of the level of light adaptation.

Sakmann and Creutzfeldt (1969) found two types. In all their units the maintained activity increased up to 10^{-3} - 10^{-2} cd/m^2; above this level some units showed a decrease in the activity, while in other units the maintained activity levelled off. It is possible that the first mentioned group in their study consisted of sustained units and the second group consisted of transient units, because the maintained activity of units of the first group diminished if steady centered spots at the same illumination exceeded the center. This was not the case in the last mentioned group.

The results in chapter 3 with respect to the maintained activity during light-on when long square wave pulses were presented and the results in section 4.3.2 indicate that the maintained activity is a measure of the strength of the surround mechanism. This has already been shown in the cat by Barlow and Levick (1969), who proved that the surround mechanism in this animal comes in at a background illumination at which the maintained activity levels off and decreases. In the Rhesus monkey this was also shown by Marrocco (1972), who found a weak surround mechanism and a high maintained activity at diffuse illumination in optic tract on-center units, but a strong surround an a low level of maintained activity of on-center units in the lateral geniculate nucleus. This means that for Tupaia, sustained units have a weaker surround mechanism than transient units in the light adapted state, since the maintained activity is high in sustained units and low in transient units. This difference in the level of the maintained activity between the two types in the light adapted state was also observed in the cat by Cleland et al. (1973).

It was found that in transient units I_t is inversely proportional to the uniform center area (4.3.3; Fig. 4.7). Enroth-Cugell and Shapley (1973b) also found an inverse proportionality of the level at which light adaptation starts, to the center area, but they do not indicate which type of unit was present. In their Fig. 5 most units have an area of 10 degrees or more, so it may be that only transient units were considered. In the sustained units of Tupaia no correlation of I_t with center size was found. It is assumed that the surround mechanism is also very important in determining I_t. Indeed units which begin to adapt at low levels have a weak surround mechanism with respect to units which begin to adapt at high levels (Fig. 4.8).

Enroth-Cugell and Shapley (1973b) found an inverse proportionality between the uniform center area and I_t only if all the background illuminations used were reduced by a factor for each unit which was equal to the sensitivity ratio between that unit and the most sensitive unit. This was done because sensitivity differences between units were attributed to factors such as physiological condition, transparency of the ocular media, location at the retina and so on. They therefore suggest an equal sensitivity in terms of dark-QSR between units. In our sample the inverse proportionality between the uniform center area and I_t was found for transient units without scaling, while the sensitivities in the dark were different (see Fig. 3.18) and could not be attributed to the above mentioned factors (chapter 3). So it seems likely that for units in Tupaia light adaptation (I_t) and dark sensitivity (QSR) are not correlated, while in the cat the 'dark-QSR' is constant (scaled) and is coupled with different values of I_t depending on the center area.

In the cat the extensive fields are favoured above small fields in the dark, because extensive fields can catch more quanta. In the light adapted state the extensive fields are saturated and the smaller, lesser adapted, units are favoured. Tupaia is an animal with a diurnal mode of life, so it is not strange that extensive quanta catching fields, which are very sensitive, were not found. So the constant 'dark-QSR' as seen in the cat is not needed.

In the cat adaptation starts at about 10^{-5} cd/m^2 (Sakmann and Creutzfeldt, 1969). In Tupaia, however, light adaptation starts between 10^{-2} - 10^{-0} cd/m^2, a difference of 3 - 5 decades, but the real difference is in fact greater, because the eye of Tupaia is smaller than the eye of the cat. Our psychophysical experiments with the same optical device revealed light adaptation of the fovea beginning at about 4.10^{-1} cd/m^2, which is in the Tupaia range, which received about one decade more light per unit retinal area because of its smaller eye diameter. Pupil areas are neglected in these comparisons, because little effect can be expected from corrections in these wide ranges. The late start of light adaptation in Tupaia does show the insensitivity of the cone system.

The results of measurements of the maintained activity in the dark suggests some possibilities for an explanation of its generation. It was found that in sustained units a strong negative correlation (-0.9) exists between the level of maintained activity in the dark and I_t (Fig. 4.9). There is also a correlation between I_t and the strength of the surround mechanism. The maintained activity in the dark in these units can be understood by the generation of activities by both the center mechanism and the surround mechanism, which are summed at the ganglion cell level. In transient units the situation is somewhat more difficult. The correlation between maintained activity in the dark and I_t is small (0.3), but positive (Fig. 4.9). This would imply that the strength of the surround mechanism in contrast with the results from sustained units decreases with increasing I_t-values. But this need not to be true, because in transient units the uniform center size in particular fixes I_t (Fig. 4.7). So the situation in transient units remains open to discussion.

There are strong indications that the adaptation pool is different for the two types of units (4.3.4). Adaptation of the peripheral part of the receptive field does not change the response from the uniform center in transient units, but in sustained units an increase in this response was observed (Fig. 4.10 shows a decrease in the QSR). Sakmann et al. (1969) found no adapting effect of the surround, while Maffei et al. (1970) showed that a steady spot located in the periphery increased the response to a sinusoidally modulated spot in the center. It is possible that two populations of ganglion cells may have been sampled. In the lateral geniculate nucleus of the cat (all sustained units project to this nucleus) steady illumination of the periphery diminishes the adapting effect of steady illumination of the center. The response to a flash in the center is stronger when the surround is illuminated (Nakayama, 1971). Also in human psychophysics (Westheimer, 1967) such a region with a similar effect for cone vision called 'sensitization' has been found.

Flashing annuli yielded sustained responses during light-off when center adapting spots were used in sustained units (4.3.5). This was never possible in transient units; short transient responses were always obtained. Furthermore only in exceptional cases was the response totally absent during light-on in transient units. These results are in agreement with the results of Enroth-Cugell and Pinto (1972), who were also unable to obtain pure surround responses from their 'surround concealing' units, which were probably transient units.

Our results with center adapting spots further confirm the superposition models used (chapter 3).

Concerning the latencies, no difference was found between the two types of units. The latency was a function of stimulus intensity and not of background illumination, which is in agreement with the data of Ikeda and Wright (1972b). These authors, however, found that the latencies of very weak responses are different at different background illuminations. This was not confirmed in our study, although very low responses were present (Fig. 4.16B), but it is possible that longer sampling with very weak stimuli would yield the same results.

4.5. Summary

1. Single unit recordings were made from optic tract on-center fibres from the all-cone retina of *Tupaia*, a primitive monkey. Properties of sustained and transient units were studied in the light adapted state.
2. The Quantum to Spike Ratio (QSR), using short 10 msec pulses, increases with background illumination. The number of extra spikes in the burst was used as the response. The shape of the curves with the increment threshold as a function of background illumination was the same for transient units and sustained units using stimuli in the uniform center and stimuli which exceed this area (up to

six times) and they obey Weber's law at the highest illumination levels.

3. Light adaptation (by diffuse fields) is coupled in both types with a shortening of the burst and with a regularly increasing maintained activity in sustained units and an unchanged maintained activity in transient units. The surround mechanism (in sustained units) is strong, when the increase in the maintained activity with light adaptation is weak and this mechanism is weak when this increase is strong.

   The surround mechanism is on the average weaker in sustained units than in transient units for all background illuminations used.
4. The increasing maintained activity with background illumination in sustained units is the result of the summation of the spatially separated center and inhibitory surround mechanism. The unchanged activity in transient units is the result of summation of coextensive center and inhibitory surround mechanisms.
5. The adaptation pool in transient units coincides with the center. The adaptation pool in sustained units consists of a central region and also of a surrounding region, which is located in the receptive field periphery and which decreases the effect of the central region.
6. In transient units the uniform center area is inversely proportional to a defined level of light adaptation (I_t). In sustained units this correlation is absent, but in these units I_t is correlated with the strength of the surround mechanism as defined by the maintained activity.
7. In sustained units there is a strong negative correlation ($r = -0.9$) between the maintained activity in the dark and I_t. This correlation is positive and weak ($r = 0.3$) in transient units.

   It is concluded that both center and surround mechanisms may contribute to the maintained activity in the dark. The surround mechanism would be inhibitory with respect to the center mechanism. Differences in balance between these two mechanisms would cause the variability among units concerning this activity.
8. With center adapting spots and annular stimulation it is possible to obtain only off-responses in sustained units. This was impossible in transient units. This result strengthens the idea that center and surround mechanisms overlap completely in transient units and are spatially better separated in sustained units.

9. The supposed latency difference (chapter 3) between the center and the surround mechanism was also found for both sustained and transient units when center adapting spots were used (section 4.3.5).
10. No differences were found between the latencies of sustained and transient units. In the dark the latency is a function of effective stimulus energy. In the light adapted state latency is also a function of effective stimulus energy and independent of background illumination.
11. Optic tract units begin to adapt at a level of 10^{-2} - 10^{0} cd/m^2, a level which is 3 - 5 decades above the level in the cat. This difference is higher by a factor of about 10 because the cat has more extensive eyes than Tupaia.

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CHAPTER 5

RESPONSES OF RETINAL ON-CENTER FIBRES IN TUPAIA AND HUMAN PSYCHOPHYSICS: COMPARISONS AND CONSIDERATIONS IN THE LIGHT OF THE THEORY OF SIGNAL DETECTION

5.1. Introduction

In this chapter the results and methods of two types of psychophysical experiments in humans will be compared with the results of electrophysiological experiments in Tupaia. In particular the Increment threshold experiments, in which the increment is studied as a function of background illumination, and Intensity discrimination experiments, in which the detectability of the difference in intensity between a test stimulus and a reference stimulus is studied, will be presented.

It is of interest to know to what extent the responses from single units can explain the psychophysical relationships observed, if the same methods are used. When comparisons have to be made, it is important to select some aspects of the comparisons to be made and to neglect other ones. The detectability index or d' in psychophysics for instance, may be better on the retinal level, or not. The same dependencies on the background illumination, however, can be expected in both types of experiments. With Intensity discrimination experiments for instance problems have to be expected, because in electrophysiology the output of single units is used, while in psychophysics many units are undoubtedly involved, and the numbers are unknown. In psychophysics the Theory of Signal Detection is a frequently used method, which is, however, seldom used in electrophysiology.

From our electrophysiological experiments it is known (chapter 3: Appendix) that several types of units are present. On-center units respond to the onset of light. Off-center units respond maximally to the offset of light; on-off units respond both to the onset and the offset of light; directional sensitive units respond maximally to a moving contrast in the preferred direction. In psychophysical experiments responses to short flashes are often studied (Thijssen and Vendrik, 1971). Because we believe that the on-center units are mediating the brightness perception of these flashes comparison of the psychophysical results will be made with the behaviour of on-center units.

It is known in human psychophysics that the increment threshold increases with increasing background illumination (Bouman, 1952; Barlow, 1957 and 1958; Thijssen and Vendrik, 1971). From our experiments we know that with increasing light adaptation several parameters of the response change in the two types of units found (chapter 4); the burst duration becomes shorter and the maintained activity can increase. Also the variability of the response and of the maintained activity, is in many cases dependent on the level of light adaptation.

For small area stimulation with pulses of short duration a square root relationship between the increment and the background illumination is found in psychophysics. In electrophysiology, however, the Increment threshold curve consists of a horizontal and a transitional part directly followed by a region where Weber's law is observed, both for the rod dominated retina of the cat (Cleland and Enroth-Cugell, 1970) and the all-cone retina of Tupaia (see chapter 4). It may be that, when the noise of the responses is also considered, the relationship changes from almost linear to a square root function.

In Intensity discrimination experiments a test flash has to be discriminated from a reference flash. In human psychophysics a linear relationship between the intensity discrimination threshold and the intensity of the reference flash was found over a range of two decades (Thijssen and Vendrik, 1971) for a rod dominated area of the retina. A linear relationship over 3.5 decades was found by Mueller (1951) in the foveal region. We know that single units with their short dynamic ranges (about one decade) alone cannot explain the psychophysical results. If, however, the output of cooperating single units is considered, a linear relationship over a larger range might be found.

For our comparisons Tupaia was chosen, a quite uncommon laboratory animal for electrophysiological experiments. But this animal has certain advantages over other animals. The most important reason why we have chosen this animal is its all-cone retina. We have to work at different background illuminations and the time constant of dark adaptation of the cones is considerably shorter than the time constant of the dark adaptation in the rods (see also chapter 2). This is important because the recording-time of a single units is limited. Furthermore, the presence of one system makes interpretations easier since there are no rod-cone interactions or mixed responses.

Theory of Signal detection

In the above mentioned comparisons the variability of the responses is considered in terms of the Theory of Signal Detection (Green and Swets, 1966). It is essential to this theory that the detection of a signal is masked by noise. The detection occurs when a fixed criterium-value is exceeded. Detection can be based on the noise, because the noise will sometimes exceed the criterium-value (false alarms), but also on the neural activity caused by the signal itself (hits).

The first workers to apply this to luminance increment detection in the visual system were Tanner and Swets (1954). In a sensory detection model based on the Theory of Signal Detection it is assumed that a transducer changes the stimulus energy into neural activity, to which activity noise can be added. The resulting activity reaches a detector, which decides whether the activity is due to the signal or not.

Two models based on the Theory of Signal Detection can be considered, viz. the multirange meter model and the multiplicative noise model (Eijkman et al., 1966; Thijssen, 1969). In the multirange meter model it is suggested that the noise is added to the neural activity caused by the signal:

$$x = \alpha F(I) + \beta$$

in which the neural activity (x) is the sum of the activity caused by the signal ($\alpha\ F(I)$) and a term β representing the noise, which causes x to fluctuate. β is independent of the magnitude I of the signal.
Consequently

$$\langle x \rangle = \alpha\ F(I) + \langle \beta \rangle$$

$$\sigma_x = \sigma_\beta$$

The range-setting mechanism, which manipulates the gain α, has to maintain the x in a fixed range in this model.
In the multiplicative noise model it is suggested that the noise increases with the magnitude I of the signal:

$$x = \alpha\ F(I)$$

in which α is the fluctuating quantity.

Thus

$$\langle\alpha\rangle = \langle\alpha\rangle\ F(I)$$

$$\sigma_x = \sigma_\alpha\ F(I)$$

It is often supposed that the underlying distributions are Normal distributions. In this chapter we shall see that this assumption is not always true. Furthermore, it was found that the noise of sustained units is of a more multiplicative nature and that of the transient units of a more additive nature.

In psychophysics it is supposed that an observer can change his criterium according to which the stimuli are judged (Green and Swets, 1966). When the criterium is high a lot of neural activity has to be present to observe the stimulus. A plot of the probability on a positive answer when a stimulus is presented (hit) against the probability on a positive answer when no stimulus is presented (false alarm) at various criterium levels is called a ROC-curve (ROC = Receiver Operating Characteristic).

5.2. Methods

The same methods were used as are described in chapter 3. Recordings were made with tungsten microelectrodes from single on-center fibres of the all-cone retina of Tupaia chinensis. The animal was paralyzed and artificially ventilated with a mixture of N_2O and O_2. The stimulus duration was 10 msec. Identical stimuli were presented 30 or 50 times with a frequency of 0.3 - 1.0 Hz. Spike numbers were counted by means of an on-line connection with a computer (PDP-9). Post Stimulus Time Histograms were made and several response measures were calculated (Fig. 5.1). The horizontal broken line in this figure indicates the beginning and the end of the burst. Both times were measuerd by taking m + 2s as the criterium, in which m and s are the mean and the standard deviation of the number of spikes of the 40 bins (binwidth 5 msec) which preceed the stimulus onset.

In this chapter the term 'increment threshold' will be used. The term 'threshold' is from the point of view of the Theory of Signal Detection, in which theory no thresholds are supposed, less suitable. Yet we use it, because we want to make comparisons with psychophysics and this term is often used in that field.

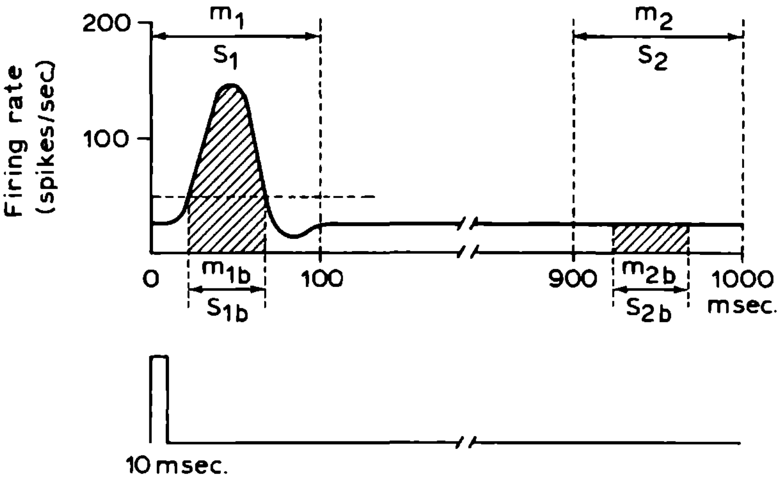

Figure 5.1 Statistical parameters calculated from a Post Stimulus Time Histogram. The dashed horizontal line indicates the criterium which will be used to define the beginning and the end of the burst (see methods).

m_1, m_2, m_{1b} and m_{2b}: mean values of spike numbers during the indicated time duration.

s_1, s_2, s_{1b} and s_{2b}: standard deviations of spike numbers during the indicated time duration.

The parameters with subscript 'b' relate to the burst duration; the other parameters relate to periods of 100 msec. So m_{1b} is the mean number of spikes and m_{2b} is the mean number of spikes of the maintained activity calculated over an interval equal to the burst duration.

5.3. Results

5.3.1. Noise in sustained and transient units

When we conceive of the pathway from the receptor cells up to and including the ganglion cell as the transducer and the number of spikes as the neural activity, we can say that the output of the transducer is linear over a limited range of stimulus intensities (chapter 3 and 4). The number of spikes, with or without stimulation, is fluctuating. So, noise

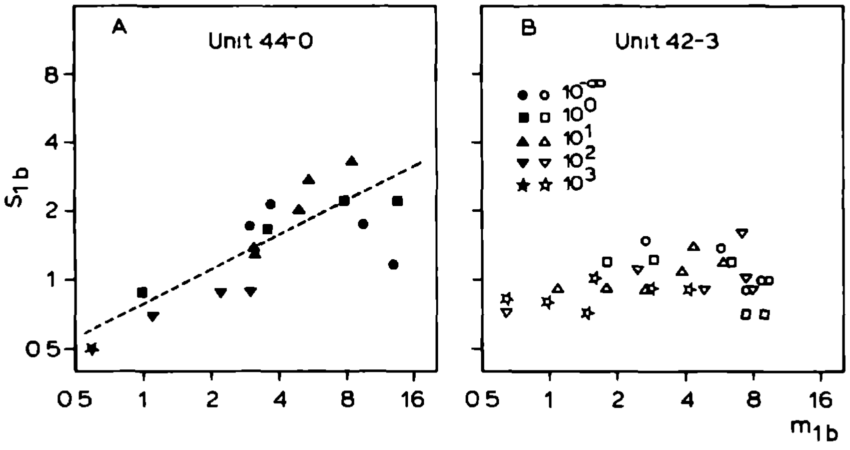

Figure 5.2. Plots of s_{1b} against m_{1b} for a sustained (A) and transient unit (B) on double logarithmic coordinates. In all cases stimuli were used; the different relative background illuminations are indicated: 10° (44-0) = 8.10^5 and 10° (42-3) = 2.10^5 equivalent quanta of 550 nm/degree^2.sec. Slope of the broken line: 0.5.

is always present. It is uncertain to what extent the variability present in psychophysical experiments is due to the noise originating in single units in the retina.

When stimuli are presented to sustained or transient units noise increases. In sustained units the noise, expressed as the variance of the number of spikes in the burst, is roughly proportional to the mean number of spikes, while in transient units this increase is considerably weaker. Fig. 5.2 illustrates this for several background illuminations for one sustained and one transient unit. A straight line (slope about 0.5) fits the data of the sustained unit up to a level of about 8 spikes, indicating a linear relationship between m_{1b} and $s_1{}^2{}_b$. In the dark this relationship is in general also present, although this cannot be seen because of the lack of points in the lower range of m_{1b}-values (Fig. 5.2). The straight line does not pass the point at which s_{1b} and m_{1b} are 1, which would be the case if $s_1{}^2{}_b = m_{1b}$ just as in Poisson distributions. The slope-value for the transient units is much lower than 0.5. In this unit s_{1b} is about 1. In Table 5.1 the slope-values are presented for 9 sustained and 14 transient units.

Unit no.	S	Unit no.	T
33-0	0.50	32-0	0.28
34-0	0.50	32-1	0.10
37-0	0.46	34-2A	0.46
41-0	0.52	35-0	0.12
43-1	0.56	35-1	0.32
44-0	0.58	35-2	0.02
45-1	0.54	36-0	0.26
46-0	0.46	37-1	0.22
56-0	0.56	38-0	0.40
		39-0	0.18
		40-0	0.04
		42-3	0.14
		58-0	0.04
Mean	0.52		0.20
St.Dev.	0.04		0.13

Table 5.1. Slope-values in the s_{1b} - m_{1b} plots (see also Fig. 5.2) for different sustained (S) and transient (T) units.

The same relationship was found if the mean (m_1) and the standard deviation (s_1) of the number of spikes in the first 100 msec after onset of the stimulus were calculated. The choice of the burst duration, in which the number of spikes was counted, is not critical as compared to the choice of a fixed period of 100 msec.

From Fig. 5.2 it can be seen that the level of background illumination does not influence the relationship between m_{1b} and s_{1b} markedly, although s_{1b}-values belonging to the highest background illuminations are slightly lower than the s_{1b}-values, measured in the dark. The sensitivity for a stimulus is, however, markedly decreased by these highest background illuminations.

It has already been found that the maintained activity as a function of light adaptation remains the same for transient units (about 1 spike/100 msec) and that this activity increases with increasing background illumination (from 1 to 4 spikes/100 msec) for the sustained units (Fig. 4.5A). The $m_2/s_2{}^2$-values remain the same (about 1) for the transient units and increase slowly from 0.8 - 1.5 for the sustained units (Fig. 4.5B). The regularity of the maintained activity increases for sustained units, because $s_2{}^2$ does not increase proportionally with the mean.

Our results indicate that the transient units show noise characteristics approximating to the noise as in the multirange meter model and that the sustained units show noise characteristics approximating to the noise as in the multiplicative noise model.

5.3.2. Pulse Number Distributions and ROC-curves

In this section Pulse Number Distributions (PNDs) of the number of spikes in the first 100 msec after onset of the stimulus and ROC-curves resulting from these distributions will be presented. The results from a sustained and from a transient unit will be shown.

Increasing intensities inside the uniform center at a constant background illumination were presented to a sustained and a transient unit and yielded each 5 PNDs (Fig. 5.3 and Fig. 5.4). PND A is the result of the maintained activity only. Poisson distributions were used to fit the found PNDs of the sustained unit, because in sustained units m_{1b} and $s_1{}^2{}_b$ are equal to a first approximation (Fig. 5.2). For the transient unit Normal distributions were used to fit the found PNDs, because the shape of these PNDs was symmetrical even at low mean values, the variances are much less dependent on the means, and in the Theory of Signal Detection, Normal distributions are believed to be frequently present.

That indeed the PNDs of sustained units show a better fit to Poisson distributions and the PNDs of transient units to Normal distributions can be seen by fitting the PNDs of both units to Poisson and Normal distributions. The chi-square values from both approximations are given as a ratio (Table 5.2).

		PND				
Unit no.		A	B	C	D	E
44-0	S	0.2	0.5	0.9	0.9	1.1
32-1	T	1.4	2.7	2.8	4.9	4.0

Table 5.2. Ratios of chi-square values of PNDs approximated to both Poisson ($\chi^2{}_p$) and Normal ($\chi^2{}_n$) distributions. The $\chi^2{}_p/\chi^2{}_n$-values of the PNDs of a sustained (S) and a transient unit (T) at different increasing stimulus intensities (PND A: no stimulus was used) are presented. See also Fig. 5.3 and Fig. 5.4.

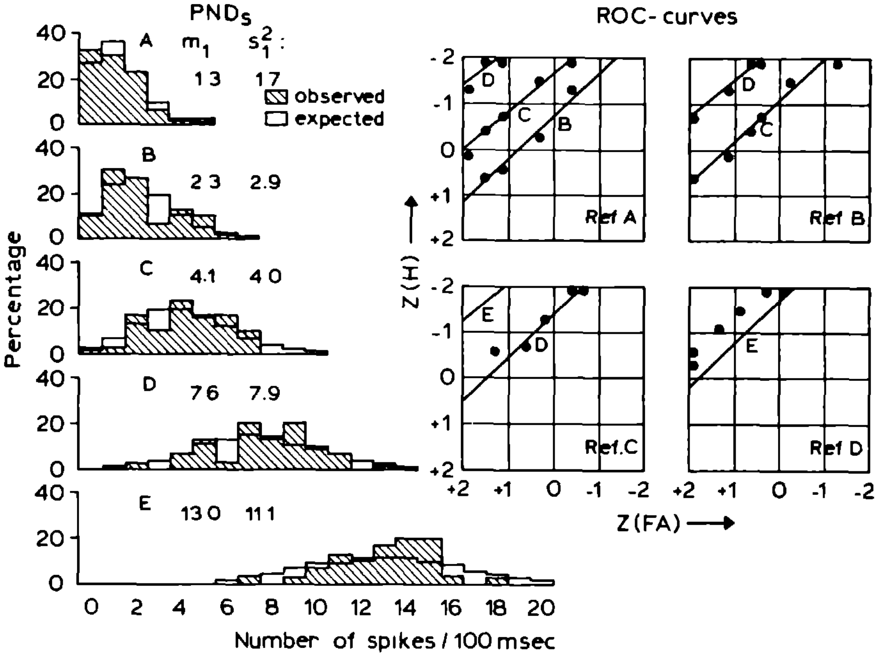

Figure 5.3. PNDs (Pulse Number Distributions) and ROC- (Receiver Operating Characteristic) curves for a sustained unit (44-0). Various increasing stimuli inside the uniform center were used (PND B, C, D and E); PND A: no stimulus was used. The observed and the expected histograms, as indicated by the m_1 - and s_1^2 - values. The fits (Poisson distribution) are in general rather good according to chi-squared tests, with the exception of PND E: A ($P < 0.50$), B ($P < 0.10$), C ($P < 0.75$), D ($P < 0.50$) and E ($P < 0.025$). Z(H) and Z(FA): double Gaussian coordinates of the ROC-curves; H: hits and FA: false alarms; Ref.: reference PND. The lines in these plots indicate the expected curves if the Poisson approximations were perfect. Background illumination: 8.10^5 equivalent quanta of 550 nm/degree^2.sec.

From this table one can conclude that the Poisson approximation is in general relatively bad for the transient unit in comparison with the sustained unit and becomes worse with increasing mean (from the left to the right in Table 5.2). This approximation is better in general for the sustained unit and is also better for the PNDs with the lowest means (A and B). The difference between the Poisson and the Normal approximation diminishes for the sustained unit with increasing mean as would be expected: the χ^2_p/χ^2_n-values tend to unity.

The PNDs of the sustained unit were approximated to Poisson distributions using the observed mean as the parameter. The PNDs of the transient unit were approximated with Normal distributions with the same parameters as those which were observed. Note that all the approximations (Fig. 5.3 and Fig. 5.4) are rather good with the exception of PND E in both units.

In Fig. 5.3 four plots with one or more ROC-curves on double Gaussian coordinates are presented. In each of these plots the reference PND used is indicated. Each PND compared with one reference PND yields one ROC-curve. An ROC-curve was constructed by determining the areas under the PND to the right of various numbers of spikes ('criterium' in psychophysics) and by expressing these in Gaussian coordinates (Z(FA) and Z(H)). The lines in these plots connect the points which would be expected if Poisson distributions were present. Note that these lines cannot be distinguished from straight lines on these coordinates, even for small mean values. The measured points fall close to the theoretical line with the exception of the ROC-curves in which PND E is concerned.

With Normal distributions the slope equals the ratio of the standard deviations. With Poisson distributions this is no longer the case. The slope of these curves does not fully reflect the differences in the standard deviations (s_1) as would be the case if Normal distributions were present. This is caused by the fact that Poisson distributions are multiplicative as well as skew distributions. The differences in slope-values (as expected with Normal distributions) decrease with increasing mean of the Poisson distribution, since the approximation to the Normal distribution then becomes better.

Fig. 5.4 shows PNDs and ROC-curves from a transient unit. PND A is the result of the maintained activity only. The standard deviations (s_1) are low and relatively constant with respect to the means. The Normal approximation to the PNDs is rather good with the exception of PND E. The observed ROC-curves agree in general with the expected curves (exception: ROC-curves based on PND E). The slopes of the ROC-curves are about unity, which would be expected with about the same standard deviations of the PNDs.

Other units were not investigated in as much detail as the two units presented in this section. It will be clear that ROC-curves do not give extra information in our study.

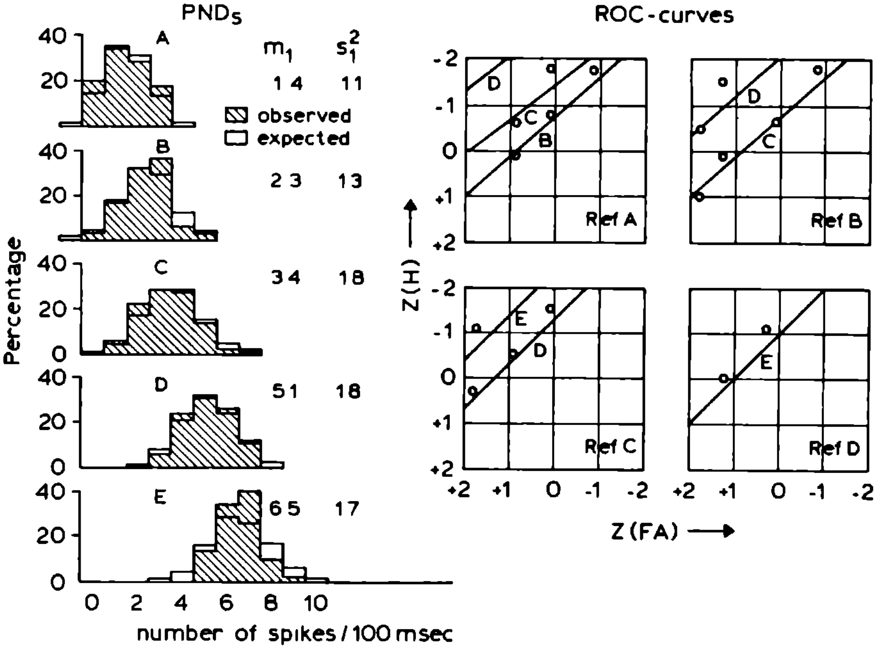

Figure 5.4. PNDs and ROC-curves of a transient unit (32-1). The PNDs were fitted using Normal distributions. P-values of the PNDs according the chi-squared tests: A ($P < 0.25$), B ($P < 0.50$), C ($P < 0.90$), D ($P < 0.75$) and E ($P < 0.025$). See also Figure 5.3. Background illumination: $1.3.10^8$ equivalent quanta of 550 nm/degree^2.sec.

The detectability index or d' is by far the most important response measure.

5.3.3. Increment threshold curves as a function of background illumination with different response measures

It is known from the literature that both in electrophysiology and psychophysics the increment threshold stays unchanged up to a certain level of background illumination and above this level increases. In psychophysics this increase is proportional to the background illumination when large and long lasting stimuli are used; with small fields and short stimuli a square root relationship is found for the rods (Bouman, 1952; Barlow, 1957; Thijssen and Vendrik, 1971). These psychophysical experiments have also been carried out for the cones (Barlow, 1968). Also in these experiments the same dependency of the Increment threshold curve on the stimulus area and the duration of the stimulus was found. For optic tract units of the cat, however, a proportionality between the Increment threshold and the background illumination is always found (Cleland and Enroth-Cugell, 1970) and Tupaia also shows this relationship (chapter 4) in spite of the use of small fields (inside the uniform center) and short pulses (10 msec).

To calculate the Increment threshold as a function of background illumination the mean number of spikes is an often used response measure in electrophysiology. In psychophysics, criteria as 'just visible' or visible in 75% of the presentations, as well as more refined criteria, which also take the variability into account (Thijssen, 1969) are used.

Several response measures can be used to calculate the Increment threshold for Tupaia. In chapter 4 the QSR (quantum to spike ratio) was used. This ratio was calculated from the number of extra spikes in a fixed interval of 100 msec. But in some cases the burst duration exceeds this interval and suppression often occurs within this period. In this section the mean number of spikes in the first 100 msec after onset of the stimulus (m_1) and the mean number during the burst duration (m_{1b}) will be used. Response measures which eliminate the influence of the maintained activity will also be used. This is done by subtracting those mean maintained activities, expressed in mean numbers of spikes during equally long periods (m_2 and m_{2b}), which preceed stimulus onset (see also Fig. 5.1). This results in two response measures, viz.: m_1-m_2 and $m_{1b}-m_{2b}$.

From chapter 4 (Fig. 4.5B) it is known that the regularity ($m_1/s_1{}^2$) increases on the average in sustained units and it will be clear that signal detection thus becomes easier. The variability of the number of spikes will be considered by using a criterium from psychophysics, viz. the detectability index or d'. In psychophysics

$d' = \frac{m_1- m_2}{s}$, in which m_1 and m_2 are respectively the mean of the supposed Normal distribution when the 'signal + noise' and the 'noise' only are present; 's' is the standard deviation of both distributions (additive noise). A ROC-curve of both distributions is characterized by the equation:

$$Z(H) = ZFA) - \frac{m_1-m_2}{s}$$

This line intersects the diagonal Z(H) = - Z(FA) at

$$Z(FA) = \frac{m_1-m_2}{2s}$$

which is half the value of d'.

In the case of different Normal distributions, $N_1(m_1;s_1^2)$ and $N_2(m_2;\ s_2^2)$, the ROC-curve is characterized by

$$Z(H) = Z(FA)\ \frac{s_2}{s_1} - \frac{m_1-m_2}{s_1}$$

This line intersects the diagonal Z(H) = - Z(FA) at

$$Z(FA) = \frac{m_1-m_2}{s_1+s_2}$$

which value will be defined as half the value of d', when multiplicative noise is also present. So

$$d' = \frac{2(m_1-m_2)}{s_1+s_2}$$

in which s_1 and s_2 are the standard deviations of the number of spikes in 100 msec periods, following and preceding the onset of the stimulus respectively.

In most PSTHs the burst duration is shorter than 100 msec. This means that extra noise caused by spikes not belonging to the burst is taken into account. This will result in a lower detectability index. Burst duration becomes shorter with increasing light adaptation, especially in the sustained units. So the shape of the Increment threshold curve will change when, instead of the burst activity during the first 100 msec, the number of spikes occuring in the burst is used. Because of this reason the detectability index over a period as long as the burst will also be used:

$$d'_b = \frac{2(m_{1b}-m_{2b})}{s_{1b}+s_{2b}}$$

In Fig. 5.5 stimulus response curves are shown for a sustained and a transient unit with different response measures (m_1, d' and d'_b). It can be seen from this figure that the position of the curves is different and that the saturation-level is different for the different response measures. This is caused mainly by the differences of the values of m_2 and s_1 between units and as a function of background illumination. The lower part of the curves can be approximated by a straight line. These lines sometimes seem to intersect the abscissa at a positive value of the relative intensity. So there seems to be a threshold. One consequence of this will be considered in the discussion.

Fig. 5.6 shows the results for a sustained and a transient unit when the above mentioned response measures

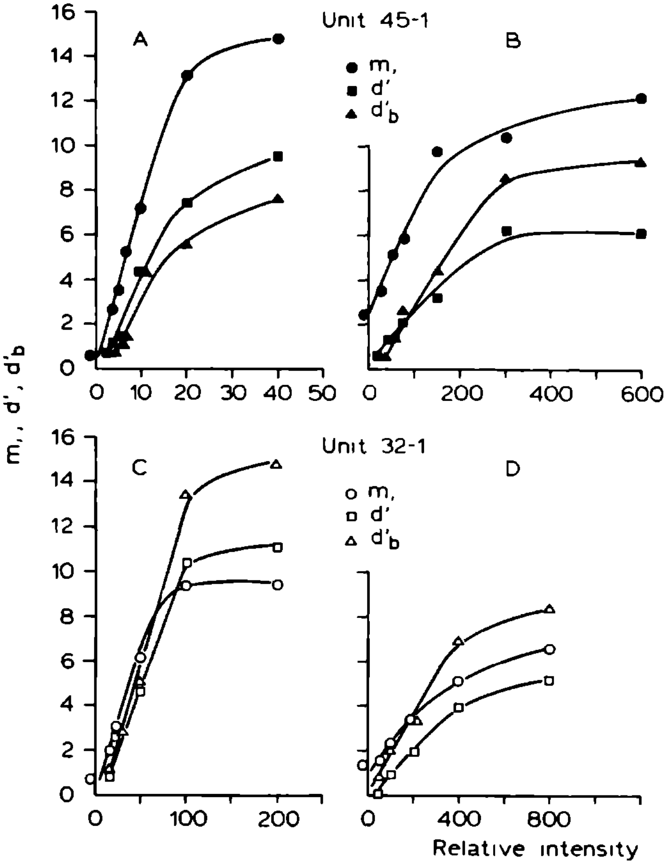

Figure 5.5. Stimulus response curves with three different response measures (m_1, d' and d'_b, see text). A and B: sustained unit; C and D transient unit. Left figures (A and C): dark adapted state; right figures (B and D): light adapted state. Stimulation (10 msec pulses) inside the uniform center. The symbols to the left of the ordinate indicate the level of the maintained activity (m_2).

are used to construct Increment threshold curves. Several units were investigated but the shape of the Increment threshold curve did not change appreciably when different measures were used. In the dark adapted state one value of the stimulus intensity was chosen. The values of the different response measures were calculated at this intensity and these values of the various response measures were used to measure the Increment threshold as a function of the background

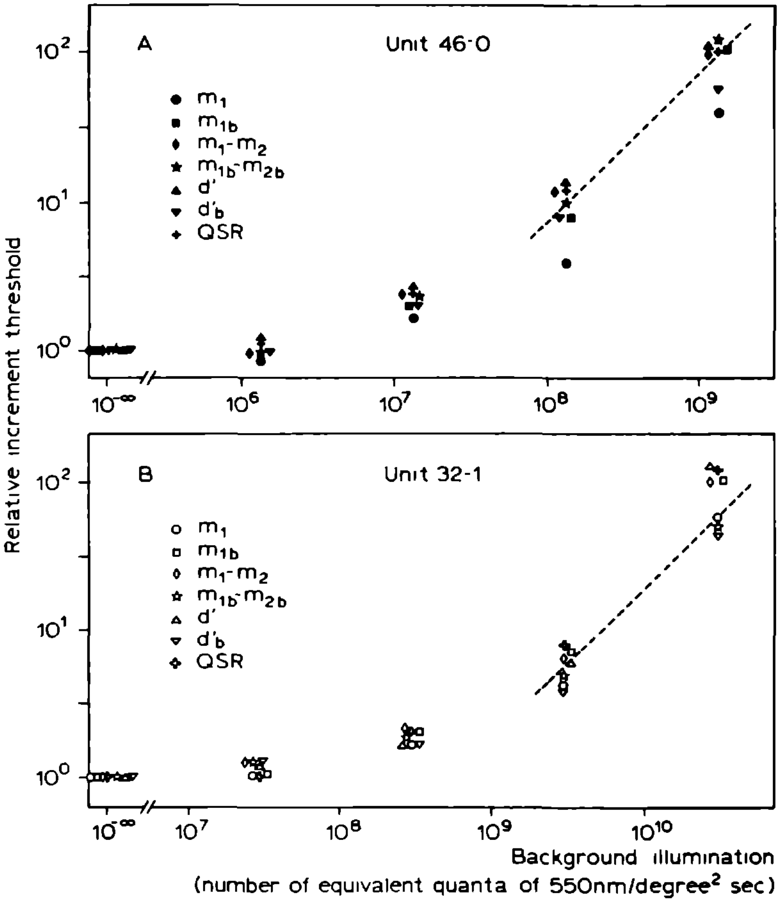

Figure 5.6. Increment threshold curves of a sustained (A) and a transient unit (B) with different response measures. A: all response measures 4.0; B: m_1 = = 2, m_{1b} = 1.6, m_1-m_2 = 1.3, m_{1b}-m_{2b} = 1.1, d' = = 1.0 and d'_b = 1.3. The broken line indicates Weber's law.

illumination. The values of the various response measures have been indicated in the legend of Fig. 5.6.

It will be clear from these data that neither the maintained activity nor the burst duration nor the variability of the maintained activity and of the activity in the burst, are sufficiently important quantities to change the curve in such a way that a long square root region would arise. In Table 5.3 the mean and the standard deviation of the slopes of the Increment threshold curves between 0.5 and 1.5 log units above the dark-QSR are shown for 6 sustained and 11 transient units with the various response measures used. It will be clear that the mean slope is independent of the different response measures. Therefore the different response measures cannot introduce a square root region into the curve.

Response measure	Sustained		Transient	
	mean	st.dev.	mean	st.dev.
m_1-m_2	0.81	0.29	0.69	0.12
d'	0.77	0.14	0.68	0.14
QSR	0.79	0.17	0.80	0.12

Table 5.3. Mean and standard deviation for the slopes of sustained and transient units. The slopes were measured from Increment threshold curves with different response measures between 0.5 and 1.5 log units above the dark-QSR. The differences between the means are not significant according to the Student t-test.

5.3.4. Intensity discrimination threshold curves as a function of the intensity of reference stimuli

In this section Intensity discrimination threshold curves will be discussed. Two light flashes were presented, viz. the reference stimulus and the test stimulus. In this type of experiment the output of single cells was used. It is assumed in psychophysics that in the case of simultaneous presentation of these stimuli the different locations are equally sensitive. Therefore we presume that for both locations identical on-center units are present.

We are interested in the question, how much extra intensity (intensity discrimination threshold) has to be added to the reference stimulus in order to reach a fixed value of a response measure within the width of the range of the reference stimulus, when the output of single retinal cells is used. Several response measures can be used (see 5.3.3.), but we use the response value, which takes into account the noise of 'both' responses, viz.:

$$d' = \frac{2(m_{1t} - m_{1r})}{s_{1t} + s_{1r}}$$

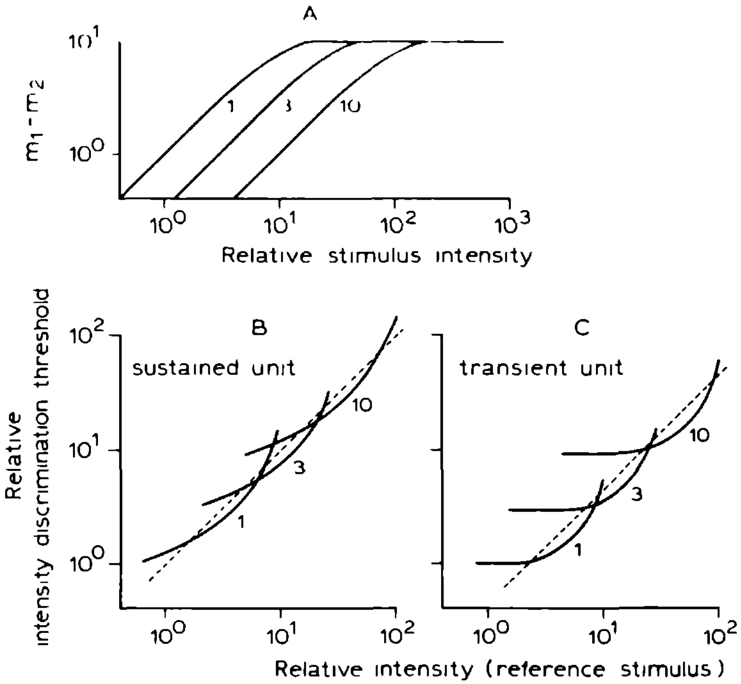

Figure 5.7. Hypthetical stimulus response curves (A) and resulting Intensity discrimination threshold curves (B, C) as a function of the intensity of a 'reference' stimulus (see text). Response measure used: d' = 1. The numbers in the figure indicate different relative QSRs (QSR-value 1 = = dark) due to different backgrounds. The broken lines indicate a linear relationship.

in which m_{1_t} and s_{1_t} represent the mean and standard deviation of the number of spikes in the first 100 msec after the onset of the test stimulus; m_{1_r} and s_{1_r} are similar measures for the reference stimulus.

In Fig. 5.7 theoretical representations of what can be expected are presented for a sustained and a transient unit. It is assumed that both the sustained and the transient units have the same stimulus response curve as shown in Fig. 5.7A. It is furthermore assumed that $s_1 = m_1^{\frac{1}{2}}$ for the sustained unit and $s_1 = 1$ for the sustained unit (see section 5.3.1., Fig. 5.2). The dynamic range is one log unit according to our experimental findings. The lower limit of this range is the intensity which yields one extra spike.

The intensity discrimination threshold of the sustained unit (Fig. 5.7B) increases roughly with the square root as long as the reference and the test stimulus are within the linear range of the stimulus response curve and increases rapidly to infinity when saturation occurs. The intensity discrimination threshold of the transient unit (Fig. 5.7C) stays unchanged in the linear range and also goes to infinity when saturation occurs. It will be clear from Fig. 5.7B and C that the range of the reference stimulus is about one log unit, just as in the case of the dynamic range of the stimulus response curve.

The same procedure has been followed for different background intensities which cause increases in QSR to values 3 and 10 times the dark-QSR; the same shapes as in the Intensity discrimination threshold curves in the dark adapted state result.

In Fig. 5.8 the measured data are presented. It can be seen that there is much scatter. Unit 33-0 and unit 42-3 resemble most the two prototypes of Fig. 5.7B and C with respect to the shape of the separate curves at each level of light adaptation. With respect to the range of the reference stimuli and the shape of the overall curves all units resemble the prototypes.

In psychophysics there is a linear relationship (Weber's law) between the intensity discrimination threshold and the intensity of the reference stimulus at one level of background illumination over several decades (Cornsweet and Pinsker, 1965; Mueller, 1951; Thijssen and Vendrik, 1971).

So it is clear from this section that the results from psychophysics are not in agreement with the electrophysiological results obtained from single retinal units.

In the discussion the assumption that summation of outputs of several single units occurs will be considered.

5.4. Discussion

It appeared that the relationship between m_{lb} and s_{lb} is different for sustained and transient units. From our m_{lb}-s_{lb} plots of sustained units it can be concluded (Table 5.1) that $m_{lb} = c.s_{lb}^2$ for different background illuminations. The constant c is different for different units. Barlow et al. (1971) found a value of about 3 for the ratio of s^2 and m (comparable measures) in the dark. This value was besides

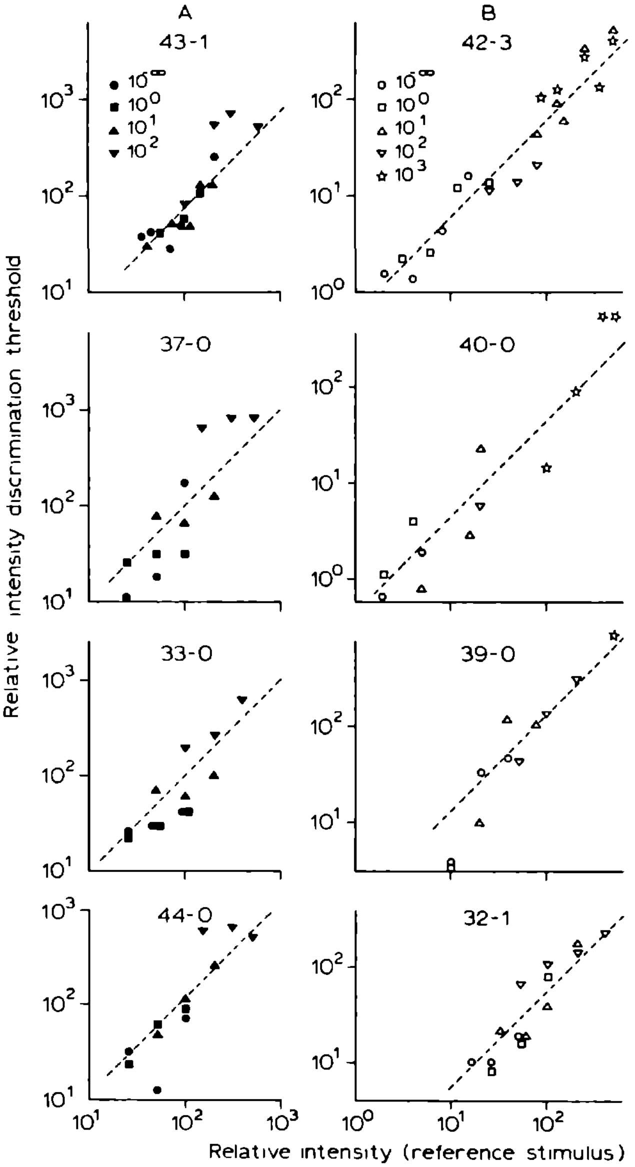

Figure 5.8. Intensity discrimination threshold curves as a function of the intensity of a 'reference' stimulus for different sustained (A) and transient (B) units. Response measure used: d' = = 1. Different symbols are used for different background illuminations. The broken lines indicate linear relationships.

other indications indicative of the number of spikes/absorbed quantum in their study. Our study indicates that the above mentioned ratio is constant in the dark and in the light adapted state. In the light adapted state certainly less spikes are generated by the absorbtion of one quantum. So the ratio of s^2 and m cannot be indicative of the number of spikes/ absorbed quantum in our study.

The fact that in Tupaia the s_{1_b} in transient units increases much more slowly as a function of m_{1_b} than in sustained units and also more slowly than in the units of the cat investigated by Barlow et al. (1971), may be partly due to the effects of the surround mechanism. In the dark adapted state the surround mechanisms of the units in the cat are not active; we have shown (chapter 3) that with center stimulation the surround mechanism of transient units is markedly stronger than that of sustained units. In order to investigate this we compared the s_{1_b} of responses from sustained units in the dark adapted state when only the uniform center was stimulated and also when a part of the receptive field periphery was stimulated. The data are presented in Table 5.4. In both situations the mean s_{1_b}-values were calculated using equal m_{1_b}-values per unit. In all units these m_{1_b}-values exceed a value of 1. In this range the s_{1_b}-values of transient units are lower than those of sustained units.

Unit no.	C	C+S
34-3	1.5	1.7
42-2	1.9	1.2
43-0	2.6	1.9
43-1	2.4	1.7
44-0	2.3	2.3
47-0	2.0	1.5
49-2	1.4	0.9
52-1	1.7	1.2
56-0	2.0	1.8
56-1	2.9	0.9
Mean	2.1	1.5
St.Dev.	0.48	0.46

Table 5.4. Mean s_{1_b}-values in the dark adapted state for sustained units. C: stimulation inside the uniform center; C+S: stimulation of the uniform center and a part of the receptive field periphery. According to the Student t-test the mean difference between the paired observations is significant ($P < 0.01$).

It appears indeed that the additional stimulation of the receptive field periphery of sustained units results in a lower value of s_{1b}.

From our ROC-curves alone one cannot conclude what type of noise, multiplicative or additive, is present, because Poisson distributions (multiplicative noise: noise = mean value) with a low mean do not clearly reflect the differences between the variances on double Gaussian coordinates. For Poisson distributions the points on the ROC-curves with double Gaussian coordinates approximate to a straight line (see Nachmias and Kocher, 1970). The slopes of the ROC-curves are smaller than unity, but do not reflect fully the ratio of the standard deviations present as in ROC-curves constructed from Normal distributions. In psychophysics slope-values of ROC-curves are indicative for the presence of additive or multiplicative noise when it is assumed that the noise is normally distributed. But it is questionable whether indeed this type of noise is present. Our results indicate that sharp distinctions between additive or multiplicative noise are not possible on the basis of ROC-curves alone, although almost pure multiplicative and additive distributions are present. The reasons for preferring the multirange-meter model, which includes the assumption of additive noise in psychophysics, are based on other arguments. Eijkman et al. (1968) presented evidence from psychometric curves that makes this preference very plausible.

A remarkable difference in the shape of the Increment threshold curves as a function of background illumination in electrophysiology and psychophysics does exist. The square root relationship between Increment threshold and background illumination, which is found in psychophysics under certain circumstances (Bouman, 1952; Barlow, 1957 and 1958 and Thijssen and Vendrik, 1971) is not found in the data from electrophysiological experiments on the cat (Barlow and Levick, 1969) and also not from Tupaia, although short pulses and small fields were used. With a different response measure, which also accounts for the variability of the responses, viz. the d', the shape does remain the same.

Our curves in which d' is plotted against the stimulus intensity on double logarithmic coordinates do not reveal straight lines in common because often these stimulus response curves seem to show a true threshold (Fig. 5.5) in the lower part of the curves. Log d' then goes to infinity when the intensity of the stimulus approximates to the 'threshold

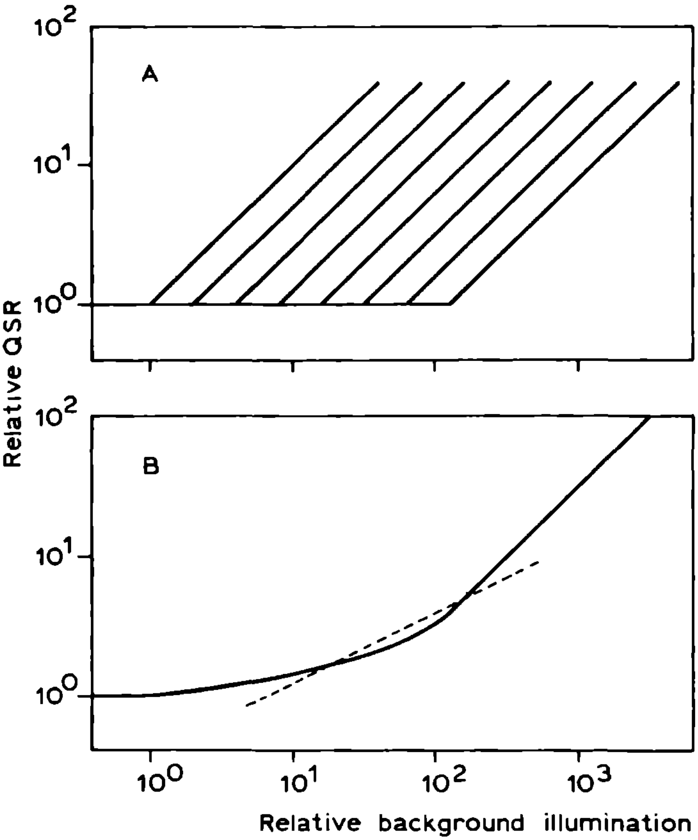

Figure 5.9. A: hypothetical Increment threshold curves for different retinal single units with different I_t-values. The corresponding stimulus response curves have the same shape as shown in Fig. 5.10A. B: Increment threshold curve of a 'higher center' cell, which averages the output of the 8 retinal units. Note that a transitional region emerges because of the different I_t-values of the units in A. The slope of the broken line is 0.5.

intensity'. Furthermore saturation occurs in the upper parts of the curves.

The middle region of these curves can be approximated to straight lines with slope-values ranging from 0.5 - 2.0. In electrophysiology Fitzhugh (1957) found slope-values ranging from 0.5 - 1.5 in the cat. In psychophysics different mean slopes were found which are dependent in part, according to Nachmias and Kocher (1970), on the stimulus conditions.

They found slopes of 1.5 in absolute detection experiments and slopes of 2.5 in Increment detection experiments. Thijssen (1969) found, as a mean of his experiments, a slope of 0.3 in the mesopical range. So also in psychophysics there is no simple relationship between the intensity and the d'.

Fischer (1973) argued that the receptive fields of retinal units overlap in the cat. It is probable that this is also the case for Tupaia. In chapter 4 it was found that different units start to adapt at different levels of background illumination. If it is assumed that in the psychophysical experiments many units are concerned and if it is supposed that these units project an a single cell of the geniculate body or another nucleus and that this unit averages the different responses, then one obtaines an Increment threshold curve with a transitional region, from which region a part can be interpreted as showing the square root relationship (Fig. 5.9). In this case no assumptions about the noise need to be made because of the independency of the shape of the Increment threshold curve of the different response measures (Fig. 5.6). A change in this transitional region of this relationship, however, does not occur when a larger area is stimulated (see chapter 4).

Other differences from the psychophysical experiments are shown by the Intensity discrimination threshold curves. In psychophysics the Increment threshold of the test flash is linear over several decades with respect to the reference flash (Thijssen, 1971; Cornsweet and Pinsker, 1965); a linear range of 3.5 log units was found by Mueller (1951) in the foveal region. Our simulation shows a different behaviour for the two types and a shorter range (Fig. 5.7 and Fig. 5.8). This discrepancy with psychophysics can be solved by assuming that more units are present. Accordingly, as the output of more units is concerned, which will be averaged in a 'higher center' cell, progessively better approximations of the slope to unity for sustained and transient units will appear. In Fig. 5.10 four such curves are shown. It is essential in this case to assume different QSRs to be present at one background illumination (see also Fig. 3.18). For the calculation the noise is also taken into consideration (see the legend of Fig. 5.10).

Although an explanation of the differences in the shapes of the Intensity discrimination threshold curves in electrophysiology and psychophysics can be found, we have not been able to find an explanation for the Increment threshold curves.

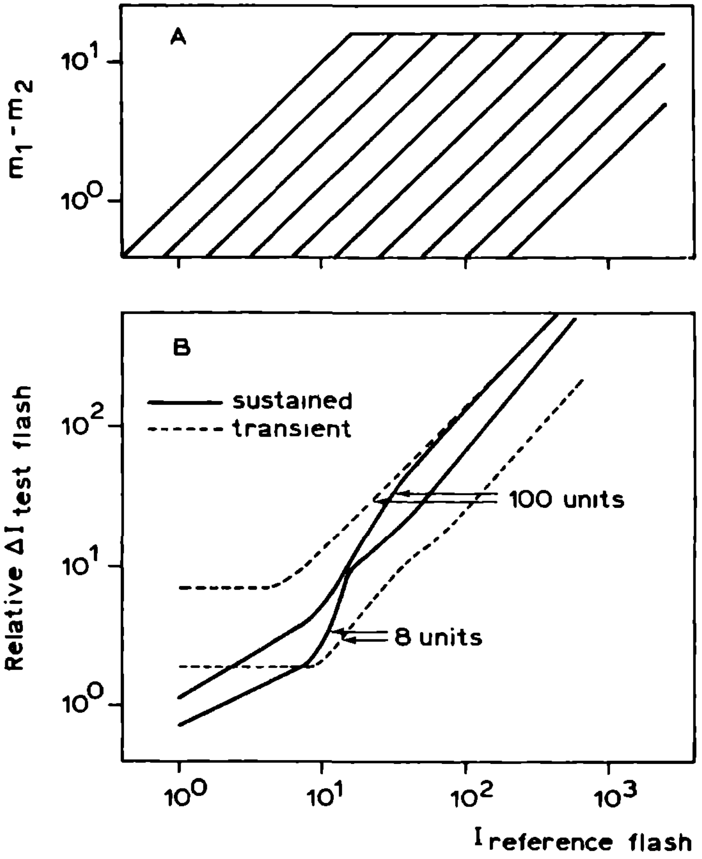

Figure 5.10. A: hypothetical stimulus response curves, which differ from each other only with respect to their QSRs. B: Intensity discrimination threshold curve of a 'higher center' cell which averages the output of 8 and 100 (see A: from the left to the right) retinal units. Response measure: d' = 1. The following assumptions were made: $s_{1t,1r} = (m_{1t,1r})^{\frac{1}{2}}$ at the retinal level for sustained units and $s_{1t,1r} = 1$ for the transient units; $s_{1t,1r} = (m_{1t,1r}/n)^{\frac{1}{2}}$ for transient units and $s_{1t,1r} = 1/(n)^{\frac{1}{2}}$ for the sustained units of a 'higher center'; n indicates the number of units averaged.

It is possible that a special class of units, which was not investigated, is involved. Indeed no units were found in the area centralis. Furthermore it is of course not necessary that higher centers only average the responses from retinal units. However, data concerning the 'higher centers' in respect to the above mentioned factors are lacking.

5.5. Summary

1. Responses of retinal on-center units of the all-cone retina of *Tupaia* were studied in relation to the results of human psychophysics.
2. The responses of sustained units are in agreement with the multiplicative noise model and those of transient units with the multirange-meter model; both models are based on the Theory of Signal Detection, which is often used in psychophysics.
3. Pulse Number Distributions of the responses of sustained units can be approached by Poisson distributions and those of transient units by Normal distribution provided that the mean is not too low.
4. From ROC-curves alone one cannot decide between additive or multiplicative noise, because of the low mean spike numbers involved in single units.
5. The psychophysically found square root relationship between the Increment threshold and the background illumination cannot be explained by features of the noise in single units of the retina.

   A linear and not a square root relationship between threshold and background illumination is present in retinal on-center units of *Tupaia*.

   When the output of many units was used a longer transitional region was found.
6. The Intensity discrimination threshold as a function of the reference flash is different in the two types of units. In sustained units there is an increase in the increment (roughly according to a square root) followed by an increase to infinity; in transient units the threshold stays unchanged and then increases rapidly to infinity.

   The range of the reference stimulus is somewhat wider than one log unit. Psychophysical experiments indicate that in both rod and cone systems the relationship is linear and shows a wider range. The linear relationship and the wider range are also found for *Tupaia* if the output of more units with different sensitivities is averaged.

5.6. References

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SAMENVATTING

In dit proefschrift zijn de resultaten vermeld van een electrophysiologisch onderzoek. Dit onderzoek werd uitgevoerd aan het visuele systeem van de halfaap Tupaia chinensis. Dit dier, afkomstig uit de oerwouden van Zuid-Oost Azië, heeft een retina, waarvan de receptoren uitsluitend of overwegend uit kegeltjes bestaan.

Evolutionair moet dit dier gezien worden als de nakomeling van een soort, waaruit enerzijds de dieren, die tot de Orde van de Insectivoren en anderzijds de dieren, die tot de Orde van de Primaten behoren, zijn ontstaan.

Het onderzoek heeft zich geconcentreerd op de retina. Vooral het dominant aanwezig zijn van kegeltjes is de reden geweest, dat dit dier als proefdier werd gekozen. Immers het kegeltjes-systeem vertoont na intense lichtadaptie een sneller verlopende adaptatie in het donker dan het staafjes-systeem en bovendien worden interacties van staafjes en kegeltjes vermeden.

De vraagstelling, welke ten grondslag ligt aan dit onderzoek, betreft de relatie tussen de electrophysiologische gegevens en bepaalde gegevens, die verkregen werden uit psychophysische experimenten met mensen. Het betreft vooral de invloed, welke ruis heeft op de gevonden eigenschappen. De term ruis heeft in dit verband betrekking op de variabiliteit van de responsies. Deze ruis is in de psychophysica niet direct meetbaar; in de electrophysiologie is deze wel direct meetbaar.

Het onderzoek werd uitgevoerd met behulp van macro- en micro-electroden. Met macro-electroden werd het electroretinogram (ERG) gemeten en met micro-electroden werden actiepotentialen van de afzonderlijke vezels, welke tesamen de Tractus Opticus vormen, afgeleid.

In dit proefschrift kan in de opeenvolgende hoofdstukken (1 tot en met 4) de weg gevolgd worden, welke bewandeld werd, alvorens een antwoord kan worden gegeven op de uiteindelijke vraag (zie boven; hoofdstuk 5). Alhoewel de verschillende hoofdstukken qua vraagstelling toewerken naar hoofdstuk 5, dragen zij, los hiervan, bij tot het inzicht in de werking van het visuele systeem.

In hoofdstuk 1 zijn de onderzoekingen beschreven, die geleid hebben tot een methode, waarmee met behulp van microelectroden stabiel van afzonderlijke vezels kan worden afgeleid. Gevonden werd, dat het anaestheticum halothaan invloed heeft op de visuele parameters en dat de narcose-diepte moeilijk zodanig te regelen is, dat bewegingen van het dier, veroorzaakt door reflexen, vermeden kunnen worden.

Het ERG vertoont onder invloed van halothaan langere piek-latenties voor zowel de a- als de x-golf dan zonder halothaan. Bovendien zijn de amplitudines voor beide golven verhoogd. Eenzelfde invloed heeft halothaan op de responsies van de afzonderlijke zenuwvezels: halothaan veroorzaakt in de tijd eerst een verhoogde vuurfrequentie gevolgd door een te lage vuurfrequentie. Dit alles heeft ertoe geleid, dat halothaan alleen gebruikt werd in de operatieve fase van het experiment. Gedurende de meetfase werd het dier verlamd met flaxedil, zodat de reflexen onderdrukt waren. Omdat het dier in deze toestand niet spontaan kan ventileren, wordt het dier kunstmatig beademd. Het gasmengsel bevat zuurstof en lachgas. Met behulp van monsters arterieel bloed werd de meest optimale ventilatie bepaald.

In hoofdstuk 2 is met behulp van het ERG en bovenvermelde methode nagegaan of de retina van de Tupaia homogeen is wat betreft de receptoren. Bepaald werden de drempelgevoeligheid in het donker, de integratie-tijd, de meest gevoelige frequentie en de zogenaamde critische flikker-fusie frequentie als functie van verschillende achtergrondniveau's en het verloop van de donkeradaptie-kromme. Gevonden werd, dat de verhouding van de amplitudines van x- en a-golf ongeveer constant is. De resultaten werden vergeleken met de in de literatuur vermelde resultaten van andere dieren en van de mens, waarbij vooral gelet werd op het type retina wat betreft de receptoren. Geconcludeerd werd, dat de retina homogeen is en vooral of uitsluitend uit kegeltjes bestaat.

De resultaten van de metingen met micro-electroden worden beschreven in de hoofdstukken 3 en 4 en gedeeltelijk ook in 5. Behalve de vezels, die responderen op het aangaan van licht (on-center vezels), werden ook andere typen vezels gevonden (appendix van hoofdstuk 3). De on-center vezels zijn uitgebreid onderzocht (hoofdstuk 3). Deze konden worden onderverdeeld in twee typen op grond van het reactiepatroon van de ontlading, wanneer een blokvormige stimulus werd aangeboden in het centrum van het receptieve veld. De twee typen, sustained en transient units, kunnen in de donker geadapteerde toestand goed van elkaar worden onderscheiden, hetgeen bij de kat zo niet onmogelijk dan toch wel zeer moeilijk is. Verschillende eigenschappen van deze vezels werden onderzocht.

Het centrum van het receptieve veld was in het algemeen kleiner bij de sustained units. De receptieve velden van de sustained units bevinden zich vooral in de buurt van de area centralis.

Tenslotte wordt voor beide typen een model voor de opbouw van het receptieve veld gepresenteerd, met behulp waarvan de meeste spatio-temporele eigenschappen begrepen kunnen worden.

De eigenschappen van de responsies van de twee typen on-center vezels in de licht geadapteerde toestand zijn beschreven in hoofdstuk 4. Gevonden werd, dat het aantal kwanten nodig voor de generatie van één extra actiepotentiaal (QSR = Quantum to Spike Ratio) toeneemt voor beide typen vezels, wanneer de intensiteit van de achtergrond verhoogd wordt. De intensiteit van de achtergrond, waarbij de QSR begint toe te nemen, werd bepaald voor de verschillende units (I_t). Gevonden werd, dat I_t omgekeerd evenredig is met de center-grootte (oppervlak) voor de transient units. De I_t voor de sustained units bleek afhankelijk te zijn van de relatieve sterkte van het surround mechanisme. Bovendien werd voor de sustained units een negatieve correlatie gevonden van de spontane activiteit in het donker met I_t.

De invloed van adapterende spots op de responsies uit het centrum van het receptieve veld werd onderzocht. De QSR nam toe als functie van de diameter van de adapterende spot (gelijkblijvende intensiteit) binnen het centrum van het receptieve veld voor beide typen vezels. Bij verder toenemende diameter van de adapterende spot daalde de QSR voor de sustained units; de QSR voor de transient units bleef nagenoeg onveranderd. Het effect, gevonden bij de sustained units, staat in de psychophysica bekend onder de naam van "Westheimer effect".

Wanneer het centrum van het receptieve veld werd geadapteerd aan licht, werden door stimulatie in de periferie van het receptieve veld responsies op het uitgaan van het licht verkregen bij de sustained units; de transient units respondeerden hierop met zowel aan- als uit-responsies. Dit is een verdere ondersteuning voor het model, gepresenteerd in hoofdstuk 3.

Tenslotte werd gevonden, dat de latentietijd van het on-center mechanisme korter wordt als functie van de stimulus intensiteit en onafhankelijk is van het niveau van de achtergrondbelichting.

De variabiliteit van de responsies van beide typen vezels is onderzocht in hoofdstuk 5. Histogrammen van de aantallen actiepotentialen op identieke stimuli worden gepresenteerd. De histogrammen, gemaakt van de responsies van de transient units, lijken op Normale verdelingen. De varianties van deze verdelingen zijn nagenoeg onafhankelijk van het gemiddelde. De histogrammen gemaakt van de sustained units lijken op Poisson verdelingen, waarbij de varianties en de gemiddelden per histogram ongeveer gelijk zijn. De variabiliteit van de responsies van de on-center vezels blijkt gecorreleerd te zijn met enerzijds het "multirange meter" model (transient units) en anderzijds met het "multiplicative noise" model (sustained units). Beide bovengenoemde modellen, van welke men in de psychophysica uit kan gaan, zijn gebaseerd op de signaal detectie-theorie.

De ROC-curves geconstrueerd m.b.v. de gevonden verdelingen vertonen voor beide typen vezels een ongeveer gelijke helling. Een verschil in de hellingen tussen de beide typen zou worden verwacht op grond van het additieve karakter van de ruis in het ene type en het multiplicatieve karakter van de ruis in het ander type vezels. Deze verwachting is echter gebaseerd op de aanwezigheid van de Normale verdeling, hetgeen niet altijd het geval is.

De vorm van de "Increment drempel" curven, waarin de increment van de stimulus is uitgezet als functie van het niveau van de achtergrond-intensiteit, blijft gelijk, zowel, wanneer het aantal actiepotentialen als responsie-maat werd genomen, als ook, wanneer hiernaast ook rekening werd gehouden met de variabiliteit van de responsie in de responsiemaat (d').

De "Intensity discrimination drempel" curven, geconstrueerd met behulp van de output van de afzonderlijke vezels, zijn verschillend voor beide typen vezels en anders dan de curven gevonden in de psychophysica. Wanneer wordt verondersteld, dat de output van meerdere vezels deze drempel bepaalt, dan worden curven gevonden, welke nagenoeg identiek zijn aan die uit de psychophysica.

CURRICULUM VITAE

Henk ter Laak werd op 19 maart 1944 te Amsterdam geboren. In 1963 behaalde hij het diploma Gymnasium-β. In datzelfde jaar begon hij zijn studie in de Biologie aan de Universiteit van Amsterdam. In 1966 werd het kandidaatsexamen afgelegd. Voor de hoofdrichting Fytopathologie werd onderzoek verricht naar het binnendringen van een schimmel en een virus in hogere planten. Als bijvakken werden Bijzondere Plantkunde, Genetica en de Mathematische Statistiek gekozen. De studie werd in 1970 afgesloten met een doktoraal-examen. In de periode juli 1970 tot augustus 1974 vond zijn promotie-onderzoek plaats op de afdeling Medische Fysica en Biofysica aan de Katholieke Universiteit van Nijmegen in Z.W.O.-dienstverband. Per 1 februari 1975 is hij bij dezelfde universiteit benoemd als wetenschappelijk medewerker van het Laboratorium voor Klinische Neuropathologie van de afdeling Neurologie.

STELLINGEN

- 1 -

De kennis van de responsies van afzonderlijke zenuwcellen is veeleer evenredig met een macht hoger dan twee dan met de tweede macht van de diameter van het cellichaam.

- 2 -

De vaak beperkte tijdsduur van een gesubsidieerd onderzoek leidt ertoe, dat nieuwe technieken t.a.v. het afleiden van zenuwcellen niet of onvoldoende ontwikkeld kunnen worden.

- 3 -

De onderverdeling van twee groepen m.b.t. de gemiddelde vuurfrequentie van de spontane activiteit als functie van de achtergrondbelichting valt waarschijnlijk samen met de onderverdeling in sustained en transient cellen.

Sakmann en Creutzfeldt (1969):
Pflügers Arch. 313, 168-185.

- 4 -

De dominante aanwezigheid van sustained cellen in de "centrale" retina en van transient cellen in de perifere retina, alsmede de verhoogde activiteit bij de sustained cellen en de nauwelijks veranderde activiteit bij de transient cellen op langdurende stimuli van constante intensiteit, wijzen erop, dat sustained cellen geschikter zijn om een constant contrast waar te nemen dan de transient cellen.

- 5 -

Het is waarschijnlijk, dat ook bij de Rhesus-aap bij afleiding van ganglion cellen met mikro-elektroden in het oog overeenkomstige typen W-cellen gevonden zullen worden als bij de kat en de *Tupaia*.

P. van Dongen; Intern rapport, 1974.
Lab. voor Medische Fysica en Biofysica,
Nijmegen.

- 6 -

De relatie tussen de intensiteit nodig voor een bepaalde responsiegrootte van het ERG bij de Tupaia en de frequentie is ingewikkelder dan die bij knaagdieren.

P. van Well; Intern rapport, 1973.
Lab. voor Medische Fysica en Biofysica,
Nijmegen.
Reuter (1972):
Pflügers Arch. 331, 95-102.

- 7 -

Het kwantificeren van gegevens verkregen uit spier- en zenuwbiopten is een noodzakelijke voorwaarde voor communicatie en samenwerking op het terrein van de pathologie van spieren en zenuwen op nationaal en internationaal niveau.

- 8 -

"Enclosed fibres" van het "witte" type spiervezels in de M. soleus wijzen op een neurogene aandoening.

- 9 -

Het nemen van spier- en zenuwbiopten is slechts dan verantwoord, wanneer de mogelijkheden voor gedetailleerd histologisch, histochemisch en submikroskopisch onderzoek aanwezig zijn.

Thomas (1970):
J. Neurol. Sci. 11, 285-295.

- 10 -

Gezien de ervaringen in het bedrijfsleven zou het aanbeveling verdienen, dat de overheid de produktiviteit van de ambtenaren tracht op te voeren door middel van arbeidstijdverkorting.

- 11 -

De lange weg, welk zal leiden tot de emancipatie van de vrouw, alsmede de vele jaren, die nog nodig zullen zijn, voordat het metro-net in Amsterdam zal zijn voltooid, maken begrijpelijk, dat het Jaar van de Vrouw en het 700-jarig bestaan van de stad Amsterdam samenvallen.

De opening van het Erasmusgebouw, alsmede de openstelling van de direkte verbinding tussen de afdelingen Oogheelkunde en Neurologie zijn sprekende voorbeelden van de mogelijkheden, welke de Universiteit van Nijmegen haar medewerkers biedt, zich adequaat op de Vierdaagse voor te bereiden.

29 mei 1975

H.J. ter Laak