Processing of transient stimuli by the visual system of the rat

Submitted to the University of Cape Town in fulfilment of the requirements for the degree MSc(Med) in Physiology

Prakash Kara **BSc, BSc(Med)(Hons)**
Neurophysiological Laboratory
Department of Physiology
University of Cape Town

August 1992

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

University of Cape Town

CONTENTS

Acknowledgements
Declaration
Abstract

(1) General Introduction

1.1. Background to this study 1
1.2. Objectives and scope of this project 3

(2) Literature review: Lateral Geniculate Nucleus

2.1. Introduction 5

2.2. Neuronal composition of the LGN and its associated nuclei 6
2.2.1. The relay cells 6
2.2.2. The interneurones 8
2.2.3. The extrinsic afferent inputs to the LGN 13

2.3. Physiology of the LGN and its associated nuclei 14
2.3.1. Receptive field properties 14
2.3.2. Response to artificial and natural stimulation 15
2.3.3. Functional significance of the thalamic circuits 21

(3) Literature Review: Visual Cortex

3.1. Introduction 23

3.2. Neuronal composition and basic circuitry of the visual cortex 24
3.2.1. The spiny neurones 25
3.2.2. The smooth neurones 27
3.2.3. Afferent inputs to the visual cortex 29
3.2.4. Efferent projections 31

3.3. Physiology of the Visual Cortex 31
3.3.1. Receptive field properties 31
3.3.2. Response to flash stimulation 35
3.3.3. Response to electrical stimulation 36
3.3.4. Neural network models 40
3.3.5. Canonical model 43

(4) General Methods

4.1. Animal preparation, anaesthesia and maintenance 49
4.2. Stereotaxic procedures 52
4.3. Electrophysiological recording and data acquisition 52
4.4. Flash stimulation technique 55
4.5. Electrical stimulation technique 57
4.6. Data analysis 57
4.7. Histological procedures 58

(5) Results and Discussion - Part 1
Cortical response to transient stimuli

5.1.	Introduction	61
5.2.	Methods for optimal positioning of the cortical recording electrodes	62
5.3.	Methods for optimal positioning of the LGN stimulating electrodes	63
5.4.	General characteristics and observations	64
5.5.	Response to electrical stimulation	65
5.6.	Response to strobe-flash stimulation	71
5.7.	Discussion	92
5.7.1.	General	92
5.7.2.	Comparison of the present data with previous studies	93

(6) Results and Discussion - Part 2
Thalamic response to transient stimuli

6.1.	Introduction	97
6.2.	Methods for optimal positioning of the LGN recording electrodes	97
6.3.	General characteristics and observations	98
6.4.	Response to strobe-flash stimulation	99
6.5.	Discussion	114
6.5.1.	General	114
6.5.2.	Comparison of the present data with earlier studies	114

(7) General Discussion

7.1.	Advantages of using transient as opposed to sustained stimuli	117
7.2.	Difference in the cortical and thalamic response to transient stimuli	119
7.3.	Difference in the flash verses electrically evoked cortical response	122
7.4.	Cortical sensitivity to changes in surround illumination	126
7.5.	Origin and mechanism of the thalamic inhibition	127
7.6.	Direction for future work	128

(8) Conclusions 130

References 131

Appendix 138

ACKNOWLEDGEMENTS

Prof. P.C. Belonje: for providing the excellent research facilities at the Department of Physiology.

Prof. R.J. Douglas: for providing unprecedented intellectual stimulation, sagacious criticism, and supervising the project from both the southern and northern hemispheres.

Dr. L.A. Kellaway: for providing technical assistance, editorial comment, and supervision.

Dr. P.K. Janicki: for continuous encouragement, creative discussions, and editorial comment.

Prof. L.C. Isaacson: for insightful criticisms and encouragement.

Dr. H.D. Einhorn: for his advice on illumination technique and measurement.

Mrs. B. Young: for immaculate assistance with histological procedures.

My family: for their loving support.

I am grateful for the following scholarship support that I received during my registration for the MSc(Med) degree in Physiology:

Medical Research Council Masters Scholarship,
UCT/Mellon Foundation Scholarship,
Manuel & Luby Washkansky Scholarship,
German Academic Exchange Service Scholarship.

DECLARATION

I, **Prakash Kara** hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the University of Cape Town to reproduce for the purpose of research, either the whole or any portion of the contents of this thesis in any manner whatsoever.

28th August 1992

ABSTRACT

While three decades of intensive cortical electrophysiology using a variety of *sustained* visual stimuli has made a significant contribution to many aspects of visual function, it has not supported the existence of intracortical circuit operations in cortical processing. This study investigated cortical processing by a comparison of the response of primary visual cortical neurones to *transient* electrical and strobe-flash stimulation.

Experiments were performed on 74 anaesthetised Long Evans rats. Standard stereotaxic and extracellular electrophysiological techniques were employed. Continuous (on-line) raster plots and peri-stimulus time histograms (PSTHs) of the extracellular spikes from 81 visual cortical and 55 lateral geniculate nucleus (LGN) neurones were compiled. The strobe-flash stimuli (0.05 ms) were applied to the contralateral eye while the monopolar or bipolar electrical stimuli (0.2 ms, 80-400 μA) were applied to the ipsilateral LGN.

60 of the 81 (74%) tested cortical units were found to be responsive to visual stimuli. A distinct and consistent difference in the cortical response to the two types of transient stimuli was found:

(a) Electrical stimulation evoked a prolonged period (197 ± 61 ms) of inhibition in all cortical neurones tested (n=20). This was the case even in those cortical units that were completely unresponsive to visual stimulation. The protracted inhibition was usually followed by a 100-200 ms phase of rebound excitation.

(b) Flash stimulation evoked a prominent excitatory discharge (5-30 ms duration) after a latency of 30-60 ms from the onset of the stimulus (n = 59). This was followed by either moderate inhibition or return to a firing rate similar to control activity, for a maximum of 40 ms. Thereafter, cortical neurones showed a sustained increased level of activity with superimposed secondary excitatory phases. The duration of this late re-excitatory phase was 200-300 ms. In 17 of 20 (85%) tested units, the temporal profile of the cortical response to flash stimulation was modulated by small changes in the level of background illumination. In 16 of the 17 units, this sensitivity was reflected primarily as an emergence of a brief *secondary* inhibitory phase at the lowest level of background illumination (0 lux). Only 1 of the 17 cortical units displayed a flash-evoked *primary* inhibitory phase at 0 lux.

We explored the possibility that neurones in the lateral geniculate nucleus (LGN) of the thalamus were responsible for the late phase of cortical re-excitation. 49 of the 55 (89%) LGN neurones could be classified as either of the "ON type" i.e. excited by visual stimuli, or the "OFF type" i.e. inhibited by visual stimuli. The response of ON-like LGN neurones to strobe-flash stimulation of the contralateral eye was characterised by a primary excitatory or early discharge (ED) phase after a latency of 25-40 ms. Thereafter, a 200-400 ms period of inhibition was observed. In 57% of the sample, a rebound excitatory or late discharge (LD) phase completed the response. OFF-like

LGN neurones were inhibited by the strobe-flash stimuli after a latency of 30-35 ms. This flash-evoked inhibition was maintained for 200-400 ms. The sensitivity of the flash-evoked LGN response to the level of background illumination was tested in 11 ON-like and 10 OFF-like neurones. No sustained secondary excitatory events, as observed in visual cortical neurones, were found in any of the ON- and OFF-like LGN neurones, irrespective of the level of background illumination.

In conclusion, the data show that the late re-excitatory phase evoked in cortical neurones upon strobe-flash stimulation, is not due to sustained LGN (thalamic) input. Rather, it suggests that these re-excitatory phases are due to intracortical processing of the transient stimuli. These findings emphasize the independent role of the cortex in computing the response to visual stimuli, and cast doubt on traditional theories that have emphasised the role of the thalamus in shaping cortical responses. The difference in the flash and electrically evoked cortical response suggests that even though substantial inhibition is available to the cortex, only a small fraction of this inhibitory capacity is utilised during natural stimulation.

Chapter 1

GENERAL INTRODUCTION

1.1. Background to this study

The neocortex plays a central role in "high level" functions such as speech production and comprehension, visual perception, and various motor skills (Polyak, 1957; Douglas & Martin, 1990). Barlow (1985) has boldly stated that neocortex is responsible for humanity's dominance of the natural world, especially for the intellectual pre-eminence that underlies this position. The apparent structural uniformity of cortex, not only in all mammals but also across different cortical areas (e.g. visual cortex, somato-sensory cortex and motor cortex) of any one mammal has been well documented (Lorente de No, 1949; Creutzfeldt, 1977; Powell, 1981; Eccles, 1984; Stevens, 1989). As a result, some have speculated that neocortex performs the same basic operation everywhere (Barlow, 1985; Martin, 1988a). The many functionally distinct areas (e.g. auditory, motor and visual cortex) are thus suspected to arise not from different operational circuits in different cortical areas but rather upon the origin of the input fibres and the destination of the output fibres (Barlow, 1985; Martin, 1988a). So the general view today, is that the neocortex is composed of a series of basic circuits (or modules) that are repeated throughout the cortex. It turns out that of all the various cortical areas studied, the *visual* cortex in particular has received the greatest attention from anatomists and electrophysiologists. As a result, a rich source of microanatomical and electrophysiological data exists for the visual cortex that outstrips by far, such detail in any of the other cortical areas investigated (Douglas & Martin, 1990). Consequently, modern scientists attempt to understand how the circuitry of the visual cortex operates, with the goal of opening the door for understanding overall cortical function.

From the above discussion, it is not surprising that physiologists have invested at least three decades of experimental and theoretical work in an attempt to understand how cortical neurones process and synthesize the raw material provided by the thalamus to result in perception. Yet, *apparently* simple neuronal operations like the generation of various receptive field properties of individual cortical neurones (e.g. orientation and direction selectivity) from non-orientation and non-direction selective thalamic neurones is still a matter of controversy. The surge of interest in the

processing ability of the visual cortex in particular, effectively started with the studies of Hubel & Wiesel (1959, 1961, 1962). Their studies involved the mapping of detailed receptive field properties of neurones in the visual pathway from the retina (mini-brain), through the thalamic nuclei (relay station), and finally to the visual cortical areas. Cells in different parts of the visual system have different receptive field properties, e.g. circular-surround receptive fields in the retina and the lateral geniculate nucleus, and elongated receptive fields in the visual cortex. Purely from their receptive field studies, Hubel & Wiesel proposed that the convergence of a row of geniculate cells was the basis of orientation selectivity in cortical cells. Hubel & Wiesel had no anatomical evidence to support the existence of multiple thalamic inputs converging onto single cortical cells. No consideration was given to the temporal pattern of discharge of the thalamic and cortical neurones. Even though Hubel & Wiesel's model of orientation selectivity is difficult to test experimentally, it remains ingrained in modern textbooks of neurophysiology (Carpenter, 1990; Mason & Kandel, 1991).

A novel approach to understanding the circuitry of visual cortex (or neocortex in general) comes from studies by Douglas *et al* (1989) and Douglas & Martin (1991). They used electrical pulse stimulation of the geniculo-cortical afferents, and horseradish peroxidase labelling of single neurones to develop a basic model of cortical computation. Their intracellular recordings revealed an unexpectedly long (300 ms) hyperpolarising inhibitory phase preceded by brief excitation in the response of striate cortical cells to pulsed electrical stimulation of the geniculocortical afferents. This, together with their pharmacological manipulation and computer simulation studies, led Douglas & Martin to conclude that the intracortical circuitry and not the geniculate (thalamic) input was responsible for the extended cortical response. This finding is not consistent with theories (e.g. Hubel & Wiesel, 1962; Hubel, 1988) that emphasize the role of the lateral geniculate nucleus in shaping the response of cortical neurones. However, electrical stimulation is artificial and results in the synchronous activation of the fibres stimulated. The multiple sub-classes of retinal and geniculate neurones that project to cortex "code" specific aspects of the visual scene. Therefore, we suspect that natural (visual) stimuli are unlikely to cause synchronous activation of geniculocortical fibres, but rather provide a spatially organised and patterned input to the cortex. Consequently, the response of cortical neurones to natural stimulation may be different to that observed by Douglas *et al* (1989) for electrical stimulation.

1.2. Objectives and scope of this project

This study analyses cortical processing by recording the visual and electrical evoked responses of *single* thalamic and visual cortical neurones *in vivo*. Unlike many of the earlier studies (Hubel & Wiesel, 1959, 1961, 1962; Humphrey & Weller, 1988; Richmond, Optican & Spitzer, 1990) however, we have chosen to use natural and artificial stimuli that are much shorter than the duration of synaptic events as a probe for analysing the mechanism of intracortical processing. Specifically, transient strobe-flash and electrical stimuli (i.e. < 1 ms in duration) were used to analyse the temporal pattern of discharge of cortical and thalamic neurones over a few hundred milliseconds. The advantage of transient flash or electrical stimuli over conventional long-durationed stimuli e.g. spots, bars, checker-like blocks, and other pattern stimuli, is that it allows one to assess the performance of the circuit (here thalamic or cortical) over time, independent of any interference from the stimulus. So the approach in this study was to trigger the thalamic and/or cortical circuits with transient (or pulse) stimuli and observe the evolution of the response of individual thalamic and/or cortical neurones embedded in these thalamic and/or cortical circuits.

Consequently, the following questions were addressed in this project:

Firstly, could the protracted inhibitory events in response to electrical stimulation, as observed by Douglas *et al* (1991) in cats, also be found in rats?

Secondly, could such sustained inhibitory events be as a result of specifically using transient electrical stimuli, or might they be reproduced with transient flash stimuli? This was determined by directly comparing the response of individual cortical neurones to electrical and flash pulse stimulation.

Thirdly, what was the degree of dependence of the cortical response to flash stimulation (whether that response be inhibitory, excitatory, or a combination of both) on the thalamic input. In other words, were thalamic neurones (which feed their input to the cortex) responsible for generating the full-blown cortical response, or could the rich intra-cortical circuitry itself account for shaping the bounded cortical response? This was assessed by recording the response of thalamic neurones to flash stimulation, and comparing them to the flash evoked response of cortical neurones.

No previous study has compared the response of cortical neurones to transient electrical and strobe-flash stimulation. Furthermore, no previous study has compared the response of thalamic and visual cortical neurones to strobe-flash stimuli. In this regard, the objectives of this study were to show the extent to which thalamic and intracortical sources contribute to shaping cortical responses to natural stimuli. In addition, the work is expected to provide insight into the nature of the thalamic input to cortical neurones. The *overall* goal of this research project was to make a contribution to the growing understanding of cortical processing at the microcircuit level. Only when we have an understanding at this level, can we address the mechanisms of specific neuronal computations like direction and orientation selectivity.

Chapter 2

LITERATURE REVIEW

LATERAL GENICULATE NUCLEUS

2.1. Introduction

With the exception of olfactory signals, all sensory information is relayed through thalamic nuclei before it reaches the corresponding cortical areas for further processing (Singer, 1977; Crick, 1984; Sherman & Koch, 1986 and 1990). The lateral geniculate nucleus (LGN), which is situated in the thalamus, relays input from the retina to the visual cortex. Twenty years ago, such a definition of the role of the LGN would have satisfied most physiologists. Today, however, the LGN is considered to be much more than a simple relay station. The LGN, *together with other thalamic nuclei* e.g. the reticular nucleus of the thalamus (RNT) have been ascribed to a variety of roles which include:

(1). Modulation of thalamocortical transmission (Singer, 1977; Sherman & Koch, 1986).
(2). Mediator of selective attention (Singer, 1977; Crick, 1984; Martin, 1988c).
(3). Gain control (Sherman & Koch, 1990).
(4). Controller of thalamic rhythmic activity in relation to the sleep-wake continuum (Singer, 1977).

Ascribing these roles to the LGN and RNT have been from several, quite different avenues of neuroscience. Some have been morphological while others have been electrophysiological. Many of the roles ascribed to the thalamic nuclei, but especially (1) & (2) above, even though attractive, are very speculative.

The dorsal division of the lateral geniculate nucleus (dLGN), which is part of the dorsal thalamus, projects *directly* to cortex and other thalamic nuclei e.g. RNT. The ventral division, which is part of the ventral thalamus, also receives retinal input, but it projects only subcortically, especially to the midbrain (Sherman & Koch, 1990) and will not be considered here.

Much of our understanding of the visual system comes from studies using rats and especially cats. The experimental animal used for our series of experiments in this study was the Long-Evans rat. Where possible, structural and functional differences between the rat and cat visual systems will be highlighted in the respective sections of this chapter. The reason for including the relevant cat studies is that many of the current theories on thalamic and cortical processing arose from experiments using this animal. The discussion below is thus a composite of facts, theories, hypotheses and ideas based largely on rat and cat studies.

2.2. Neuronal composition of the dorsolateral geniculate nucleus (dLGN) and its associated nuclei

The LGN comprises of the following three components: (a). the relay cells which project to cortex, (b). the interneurones (or intrinsic neurones), and (c). the extrinsic afferent inputs to the nucleus.

2.2.1. *The relay cells*:

The relay neurones project to cortex with collateral innervation of the RNT. In the cat, the relay cells constitute approximately 75 % of the thalamic neuronal population (Sherman & Koch, 1990). Three different morphological types exist i.e. W, X, and Y cells. The X and Y types are depicted in Fig. 2.2 A & B.

The dLGN of the rat is clearly not laminated (Peters, 1985; Sefton & Dreher, 1985). The cat dLGN, however, is characteristically laminated into at least four divisions (Sherman, 1985a; Sherman & Koch, 1990). Each laminae has a different role in visual perception. This is because the cell types in each have different destinations and intrinsic morphologies. For example, the W (relay) cells are found primarily in the C laminae and project to visual areas 17, 18, and 19. The W cells have relatively small cell bodies and characteristically thin axons (Fig. 2.1 A) and their axonal arborizations are large but sparse (Sherman, 1985a; Martin, 1989 - personal communication). The Y relay cells however, are associated with all four laminations (A, A_1, C, and the medial interlaminar nucleus - MIN). Y cells have characteristically large cell bodies and thick (fast conducting) axons (Fig. 2.1 and 2.2). They, like the W cells, also project to the striate (area 17) and extrastriate (areas 18 and 19) cortices.

Their axonal arborizations are large and dense (Fig. 2.1 B). The last cell type, the X (relay) cells, are found in the A, A_1 and C laminae of the cat dLGN. They project exclusively to striate cortex, and have medium sized cell bodies and axons (Fig. 2.1 and 2.2).

The properties of these W, X, and Y cells of the dLGN are summarised in Table 2.1. (below).

Table 2.1: Main features of W, X and Y neurones in cat retinal and LGN cells (from Sherman & Koch, 1990).

Property	W cells[a]	X cells	Y cells
Receptive field size	Large	Small	Medium
Contrast sensitivity	Poor	Fair	Good
Spatial resolution	Poor	Good	Fair
Temporal resolution	Poor	Fair	Good
Axon conduction velocity	Slow	Medium	Fast
Retinal ratio	10-20%	75-80%	5-7%
LGN relay cell ratio	10%	40-50%	40-50%

[a]*Here reference is made only to the subset of W cells that appear to be involved in retino-geniculate innervation.*

The properties of the dLGN W, X, and Y cells are similar to the W, X, and Y retinal ganglion cells. Therefore the segregation of W, X, and Y pathways are maintained from the retina, through the LGN, and at least until the terminations in cortex. The main target of the X and Y cells is layer 4 of cortex, while the primary target for the W cells seems to be layer 1 (and 3) cortical cells (Fig. 2.1 B). Exactly how the X, Y and W cells "connect" to one another is not known. The segregation of these W, X, and Y inputs have led to the important concept of parallel processing (Sherman, 1985a). The W, X, and Y cell pathways (in the cat) are the basis of the parallel model for visual perception, in which each pathway analyses somewhat different aspects of the visual scene (Sherman 1985 a & b).

Unlike the description provided for the cat LGN (above), the characterisation and classification of cell types in the rat dLGN (and retina) is quite unclear. Sefton & Dreher (1985) have reviewed the physiology and anatomy of the rat visual system. The lines of evidence presented are somewhat contradictory

and confusing. All the different classifications from several authors have been given. No general consensus seems to prevail as to which of these classifications are the most acceptable.

In the rat visual system, there also appears to be a segregation of the W and Y like pathways in the retina and the LGN (Sefton & Dreher, 1985), and so supports the concept of parallel processing. Sefton & Dreher (1985) emphasized that the X pathways seen in the cat are non-existent in the rat visual system. However, there may very well be X like neurones in the rat visual system. This is because Sefton & Dreher (1985) based their classification of geniculate cells largely on Golgi staining morphological identification (Fig. 2.3), and perhaps HRP staining of rat geniculate neurones may reveal morphologies similar to that of cat geniculate neurones (Fig. 2.2).

Sefton & Dreher (1985) estimate that there is approximately only one retinal ganglion cell (RGC) for each relay cell in the LGN. This appears to be the case for rats (25 000 RGCs') and monkeys (1 million RGCs'), but not cats. In cats, there are approximately four times as many cells in the LGN as compared to the number of optic tract axons that project there. However, to pool the different classes of cells (e.g. W, X, and Y like) may be misleading. If we consider the proportions of just the Y cells in the retina and thalamic nuclei, a different picture emerges. In the cat, the percentage of Y cells in the retina and LGN are 5-7% and 40-50% respectively (Table 2.1 above). In the rat, the figures are 1% and 33% for retina and LGN respectively (Sefton & Dreher, 1985). Immediately apparent is a dramatic amplification of the Y pathway from the retina to the LGN. On the other hand, the X pathway (in the cat) converges or "shrinks" as it progresses from the retina to the LGN i.e. 75-80% and 40-50% in the retina and LGN respectively (Table 2.1). The W like pathways (in the cat) remain largely non-amplified (Table 2.1). Such details on W and X pathways for the rat remain absent in the literature.

2.2.2. *The interneurones*

Sherman & Koch (1990) estimate that 25% of the cells in most thalamic nuclei are interneurones. This figure appears to be more conservative in rats where the percentage of interneurones in the LGN is estimated to be about 7% (Sefton & Dreher, 1985). A feature of LGN interneurones is the existence of dendrites that synapse onto other dendrites. This seems to be consistent for the rat (Sefton & Dreher, 1985) and cat (Sherman & Koch, 1990). These

dendrites (of interneurones) make Gray type II (symmetrical) synapses, are GABAergic (Sherman & Koch, 1990), and therefore presumably have an inhibitory action on geniculate relay cells (Sefton & Dreher, 1985). The interneurone forms part of the geniculate glomerulus which constitutes the convergence of the terminals (appendages) of the retinal axon, the geniculate interneurone, and the relay cell (Fig. 2.5 A).

Representative geniculate interneurones from the cat and rat are shown in Fig. 2.2 C and 2.3 A respectively. Comparing these two types of interneurones, it appears as if the morphologies are quite different. i.e. the cat geniculate interneurones have long thin dendrites that form extensive dendritic arborisations while the rat geniculate interneurones also appear to be thin and long, but the dendritic arborisation seen in cat interneurones are virtually nonexistent in the rat. However, the LGN interneurone of the cat (Fig. 2.2 C) was labelled intracellularly with horseradish peroxidase (HRP) while the interneurone of the rat (Fig. 2.3 A) was Golgi stained. Thus, the morphological differences may be as a result of the different labelling techniques. The Golgi stain is regarded to be incomplete while the HRP dye provides a complete morphological representation of a particular neurone (Douglas & Martin, 1990). It would therefore be interesting to see the morphology of rat interneurones (and relay cells) labelled with HRP, and then compare them with those of the cat.

The functional similarity of geniculate interneurones and RNT cells have led some researchers to group them together as local interneurones. It is unclear whether the interneurones and the RNT have fundamentally different roles in retinogeniculate transmission (Sherman & Koch, 1990). We can however speculate on these i.e. interneurones (in the cat) are confined only to X pathways and since the geniculate X cells have good spatial resolution, the geniculate interneurones may be involved in this property of the X cells.

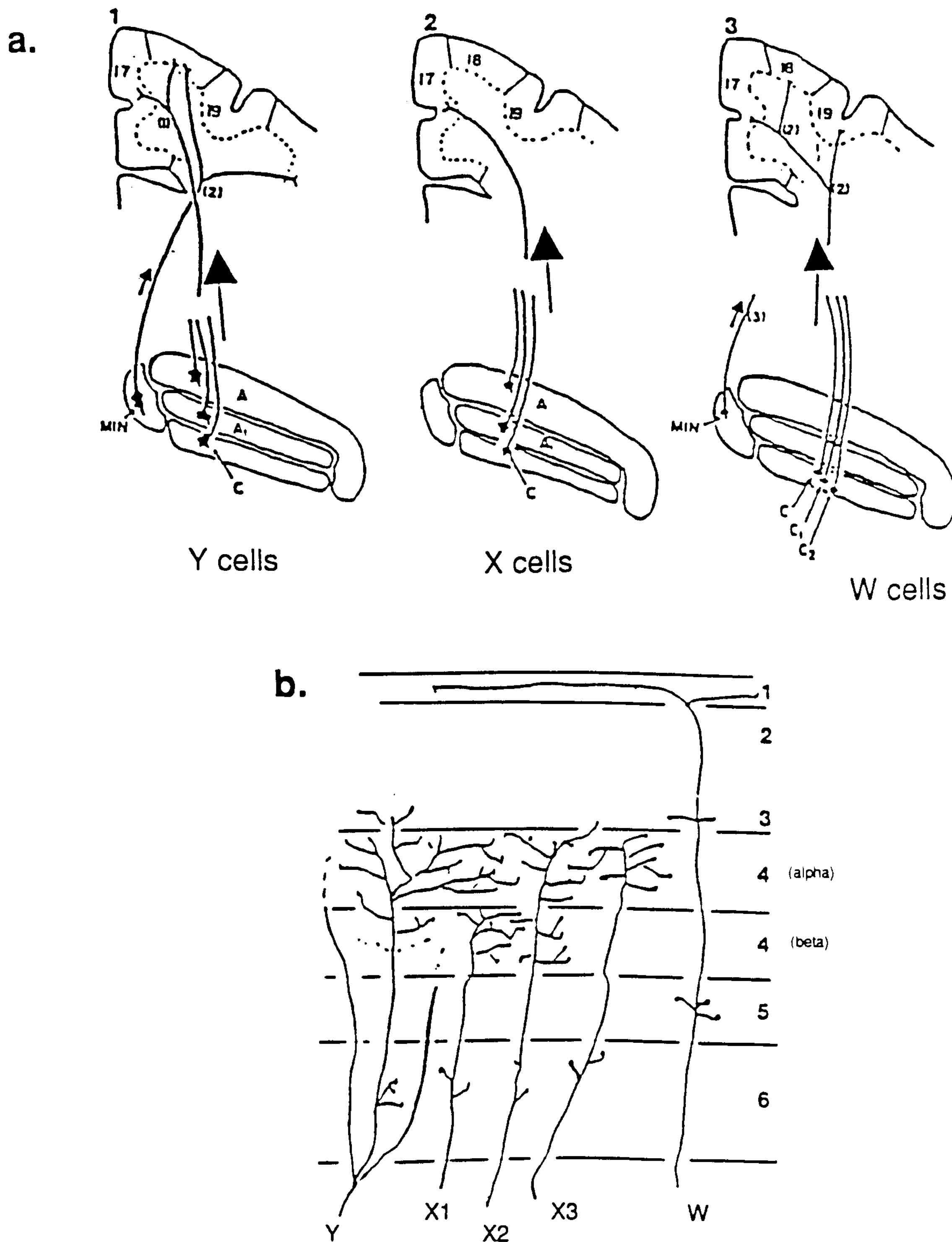


Fig. 2.1. **A.** Schematic representations of Y, X and W geniculocortical pathways in the cat. Note that the Y cells have large somata and thick fast-conducting axons. They project to striate and extrastriate areas. On the other hand, X cells have smaller somata and thinner geniculocortical axons, and project exclusively to striate cortex (area 17). **B.** Axon arborisations of geniculocortical fibres in the cat visual system. Note that the arbours of W cells are large and sparse whereas the arborisations of Y cells are large and dense. The arborisations of the X cells are small and dense (from Martin, 1989 - unpublished).

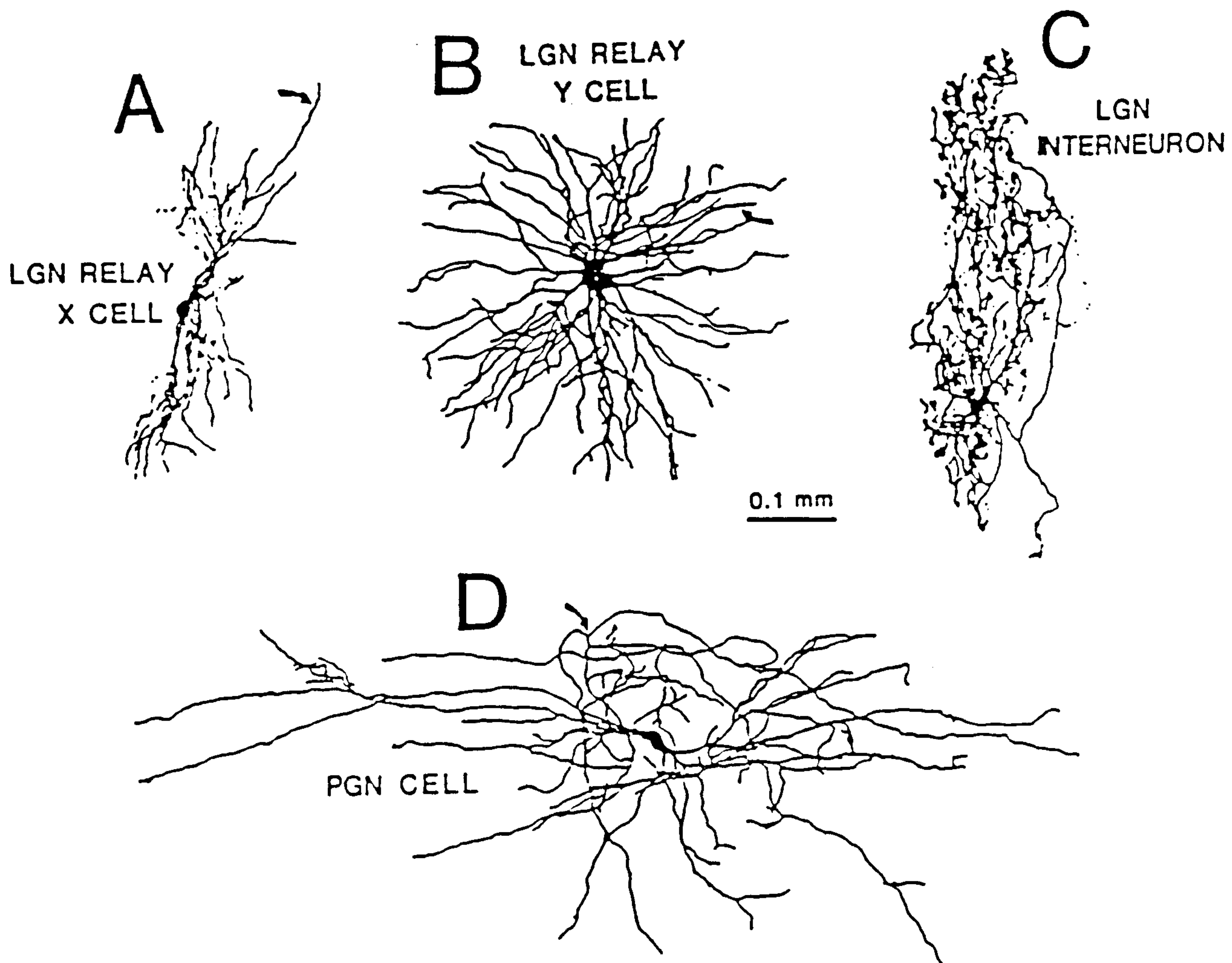


Fig. 2.2. Tracing of four representative neurones from the cat's LGN and perigeniculate nucleus, which is the equivalent of the RNT for the A-laminae of the LGN. Each cell was first studied physiologically and then labelled intracellularly with horseradish peroxidase. Where obvious, the axon is indicated by an arrow. **A**. Relay X cell; **B**. Relay Y cell; **C**. Interneurone; and **D**. Perigeniculate neurone (from Sherman and Koch, 1990).

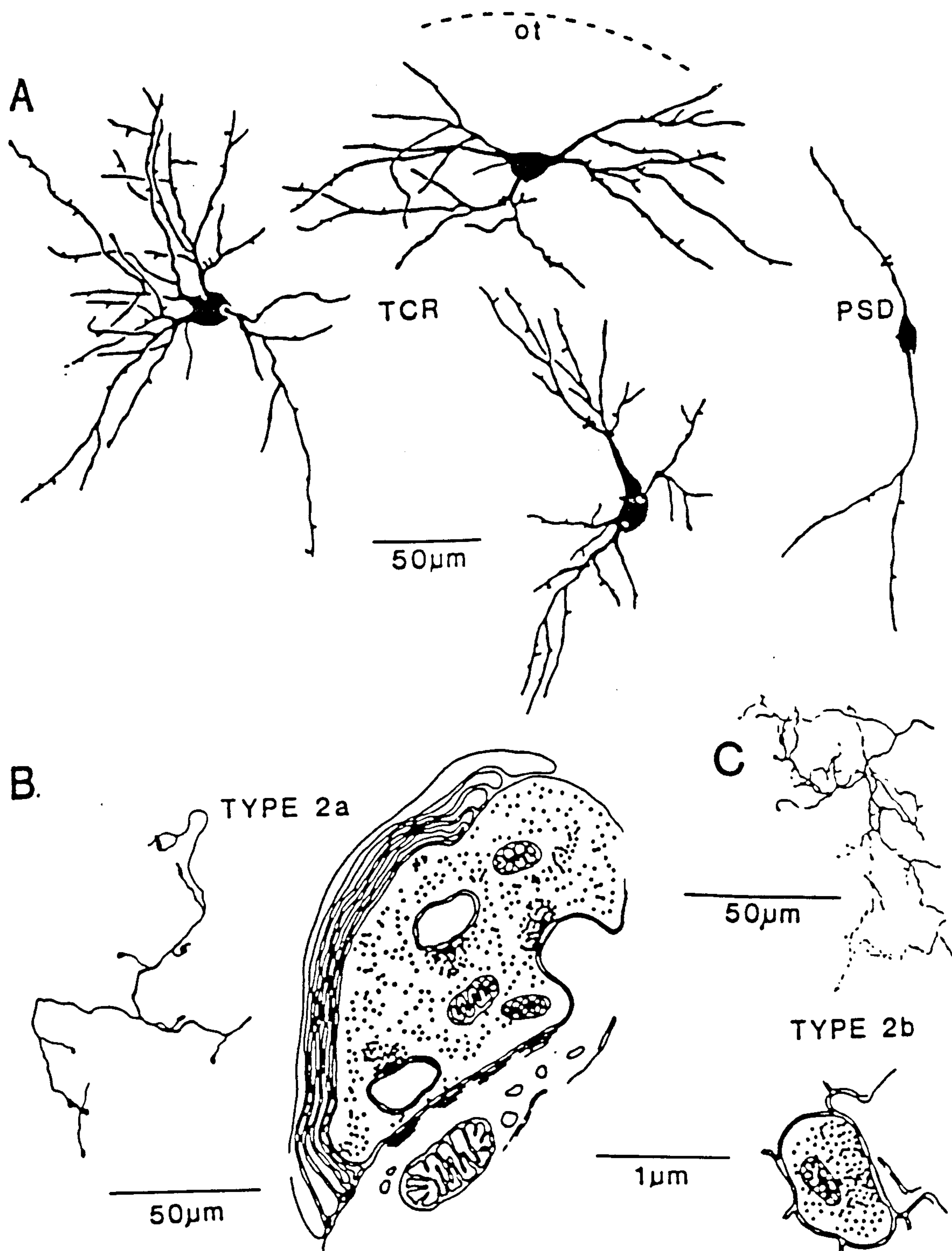


Fig. 2.3. Cells and axons in the dorsal lateral geniculate nucleus (dLGN) of the rat. **A.** Golgi impregnated neurones in the dLGN; three examples of thalamocortical relay (TCR) cells and one (PSD) cell (interneurone). **B.** Axon (Golgi material) of the larger type 2a bears on average 23 large terminal boutons, shown here drawn from an electron micrograph. **C.** The smaller type 2b axon (Golgi preparation) and terminal (electron micrograph). Note that the 2b axon ramifies more extensively than the 2a, and has more terminal boutons, on average, 160 (from Sefton & Dreher, 1985).

2.2.3. *The extrinsic afferent inputs to the LGN*

Retinal inputs

In the rat visual system, the major retinal afferents to the LGN arise from the contralateral eye. Consequently, the ipsilateral afferents provide only about 2-3% of the geniculate input in the hooded rat (Sefton & Dreher, 1985). In more "elaborate" visual systems, e.g. the cat and monkey, the percentage of ipsilateral input is greater and is related to the degree of binocularity of that species. In primates, who have the greatest level of binocularity (essential for three dimensional analysis), approximately 50% of all retinal ganglion cells project ipsilaterally (Frisby, 1979; Martin & Perry, 1988).

The number of retinogeniculate axons varies for each species and recent estimates are 25 000 for rat (Sefton & Dreher, 1985), 100 000 for cat, and 1 million for monkeys and humans (Sherman & Koch, 1990). All retinogeniculate axons, in rat, cat, and primates, are excitatory. While most of these appear to be glutamatergic, part of the retinogeniculate input is via N-methyl-D-aspartate (NMDA) receptors (Moody & Sillito, 1988).

Cortical inputs

The anatomically dominant input (extrinsic) to the LGN arises from cortex (Sherman & Koch, 1986; 1990). The corticothalamic axons originate largely from layer six cortical cells and terminate on distal dendrites of relay cells. This appears to be consistent for rat (Sefton & Dreher, 1985) and cat (Singer, 1977; Crick, 1984; Sherman & Koch, 1990). Sherman & Koch (1990) added that 50% of layer six cortical neurones contribute to the corticogeniculate pathway. However, they have not commented on the fraction of this input that is indirect i.e. via the RNT. Like the retinogeniculate axons, the corticogeniculate axons have excitatory terminations i.e. make Gray type I synapses with their postsynaptic targets and appear to be glutamatergic (Giuffrida and Rustioni, 1988). Even though the corticothalamic pathway shows considerable divergence and convergence, it conforms to the retinotopic map of the LGN. Corticogeniculate axons enhance the responses obtained from the receptive field centre, as well as reducing the activity and antagonism of the surrounds of the majority of off-centre, but only about half of the on-centre cells (Sefton & Dreher, 1985). Sefton & Dreher added that the cortical input (to the LGN) can mediate both excitation and inhibition. This

inhibition must necessarily be indirect because the corticothalamic terminals make only Gray type I (excitatory) synapses on relay cells (Sefton & Dreher, 1985; Sherman & Koch, 1990).

RNT inputs

The final extrinsic source of innervation to each of the two LGN nuclei is that from the reticular nucleus of the thalamus (RNT). The mammalian RNT is a sheet of cells that encapsulates much of the rostral and lateral surfaces of the dorsal thalamus (Jones, 1985; cited by Crabtree & Killacky, 1989). Axons of the RNT project exclusively subcortically, back into the thalamus, particularly to the nucleus that supplied the input to the RNT (Crick, 1984; Sefton & Dreher, 1985). The RNT should not be confused with the brainstem reticular formation because it (the RNT) forms a shell anteriorly and dorsally around the dorsal thalamus (Sherman & Koch, 1990). The RNT cells are all GABAergic i.e. uses gamma-aminobutyric acid (GABA) as the neurotransmitter, and synapse on apical dendrites of geniculate relay cells (see Fig. 2.5). The area of the RNT that is considered to be "visual" is commonly referred to as the perigeniculate nucleus (PGN). This seems to be consistent for cat (Sherman & Koch, 1990) and rat (Sumitomo *et al*, 1977). For the purpose of this discussion (and for the thesis as a whole) I will consider the RNT and the PGN to be one and the same area.

2.3. Physiology of the LGN and its associated nuclei

2.3.1. *Receptive field properties*

In the LGN, the same circular-surround receptive fields are found as those of retinal ganglion cells (RGCs). However, the surround inhibition in geniculate cells is stronger than that of the RGCs. This ensures that the response is weak or absent when both the centre and surround are stimulated simultaneously. These findings are consistent for rat (Sefton & Dreher, 1985) and cat (Martin & Perry, 1988; Sherman & Koch, 1990). These receptive field properties of the LGN (and the RGCs) are off-course quite different to the elongated bar-like receptive fields of visual cortical cells (Hubel & Wiesel, 1962; Shepherd & Koch, 1990).

As we shall see below, the receptive field properties of the LGN and cortex are not as simple as that described above, for some geniculate cells have recently displayed a property previously seen only in "hypercomplex" visual cortical cells (Murphy & Sillito, 1987). This property is end-stop inhibition, and requires the stimulus bar to be located within some restricted portion of the receptive field. Thus, if the bar is lengthened, the response of that particular extrastriate cortical (and now geniculate) cell becomes suboptimal. The optimality is also sensitive to the orientation of the stimulus bar (just as are the "simple" receptive fields of striate cortical cells).

A startling fact is that many of the recent reviews that discuss the physiology of the LGN do not even consider this property of end-stop inhibition in geniculate cells as described by Murphy & Sillito (1987). Since their study, there has been no evidence to suggest the contrary. Neither have there been any studies that have repeated their experiments to at least determine what percentage of geniculate neurones encountered, displayed end-stop inhibition. Martin (1988c) concluded that several circuits along the visual pathway may generate the property of end-stop inhibition. From this, we can make two conclusions i.e. either (i). Murphy & Sillito's observations are flawed or (ii). their findings are genuine but scientists reviewing the topic (LGN circuitry) tend to neglect it because the underlying principles of its origin are difficult to explain, or because it limits their data or hypotheses. Murphy & Sillito's finding is exciting because it removes the view of the thalamus being a simple way-station committed to slavery by merely directing retinal information to the relevant cortical areas. If the thalamus was indeed a simple relay station, it would be expected that the retinal inputs be fed *directly* to the cortex.

2.3.2. *Response to artificial and natural stimulation*

According to Sefton & Dreher's (1985) observations, on the basis of electrical properties, cells recorded in the dLGN have been classified into relay (or principal), and interneuronal types. Upon electrical stimulation of the optic nerve (tract), the relay cells responded with a short latency action potential followed intermittently by bursts of three to five spikes for periods of one second or more. These relay cells could also be activated by *antidromic* stimulation of the striate cortex (Sefton & Dreher, 1985).

Fukuda *et al* (1973 & 1975) have shown that upon natural (e.g. strobe-flash) stimulation of the contralateral eye, relay cells (in Albino rats) responded

with a characteristic early discharge (ED) after a latency of 38-44 ms. This is almost always followed by a long (at least 150 ms) phase of inhibition (i.e. absence of spontaneous activity). Moderate rebound excitation then usually completes the response. Fukuda *et al* classified relay (or principal) cells into slow and fast types (see Fig. 2.4 A and B), and so attempted to equate them to the X and Y cell types found in the A laminae of the cat LGN. This classification seems inappropriate as there is a huge overlap of the latencies between the two classes of principal cells recorded by Fukuda *et al* (1973 & 1975). The overlap between their two classes of relay cells was greater for the latencies to flash stimulation (of the eye) as compared to the latencies upon electrical stimulation (of the optic tract).

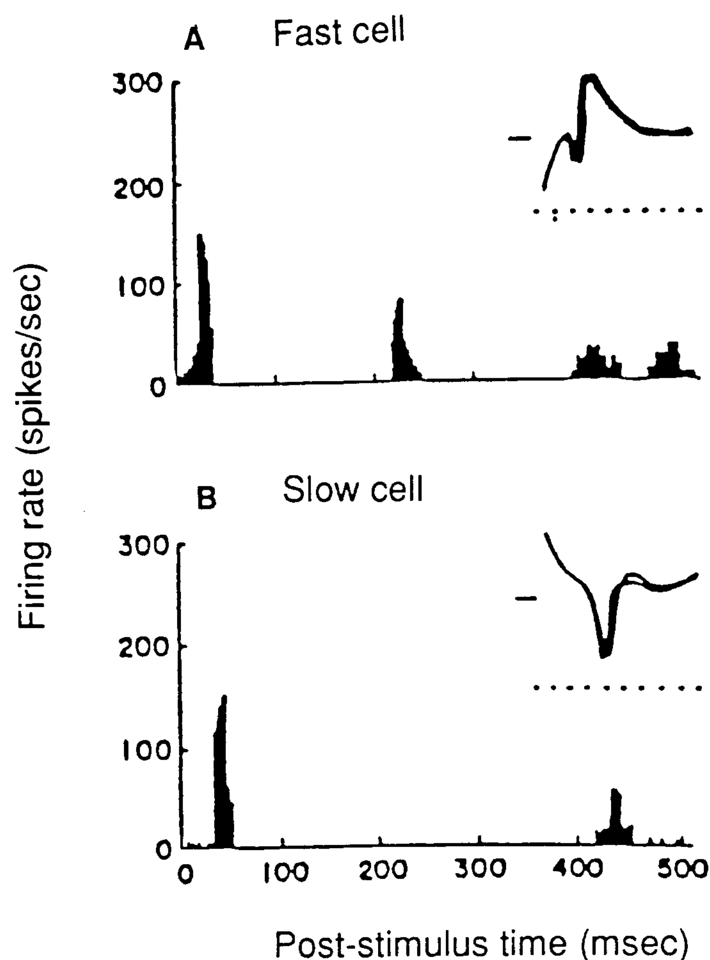


Fig. 2.4. PSTHs of a "fast" (**A**) and "slow" (**B**) cell in the rat lateral geniculate nucleus (LGN) upon flash stimulation with maximal intensity. Insets are field potentials of LGN cells upon electrical stimulation of the optic tract (time scale = 1 ms per division). The latency is 1.8. ms in insets (A) and 3.1 ms in (B). See text for further details (from Fukuda *et al*, 1975).

From the data presented above, it is difficult to assess whether there are truly functionally different classes of geniculate cells in the rat visual system. Support for functionally different classes comes largely from morphological studies i.e. W, X and Y like cells (as discussed in section 2.2 above).

I will now briefly discuss the possible origin of the post early discharge (ED) inhibition that is usually seen in geniculate cells upon flash stimulation (e.g. see Fig. 2.4).

The early studies (e.g. Burke & Sefton, 1966; cited by Sefton & Dreher, 1985) suggested that the post ED inhibition seen in relay cells was mediated by the interneuronal cells. This was because the interneuronal cells responded (disynaptically) with a burst of up to 12 spikes upon optic tract stimulation. However, more recently, it has been found that these interneuronal cells lie outside the LGN, in the reticular nucleus of the thalamus (RNT) (Sefton & Dreher, 1985).

Considering the basic circuits proposed by Sherman & Koch (1990) (see Fig. 2.5), it is not known exactly which of the different synaptic inputs from cortex, RNT, and local interneurones, mediate the post ED inhibition, or any of the other inhibitory actions on geniculate relay cells. As far as the post ED inhibition is concerned, one could speculate that extrinsic inputs from the RNT and cortex *initiate* the geniculate inhibition. The inhibition could then be *maintained* by either:
(1) Synaptic mechanisms via $GABA_B$ receptors which are known to have long time-constants (Crunelli & Leresche, 1991), or
(2) Intrinsic electrophysiological currents e.g. IK_{Ca} - a calcium dependent potassium current. While such intrinsic inhibitory conductances are known in great detail (Crick, 1984; Sherman & Koch, 1986 & 1990), exactly how they influence the response of geniculate relay cells is only speculative and are considered later in this chapter, or
(3) A combination of both synaptic and intrinsic mechanisms (Crunelli & Leresche, 1991).

Hull (1968), using monkeys, and Kalil & Chase (1970), using cats found that cooling cortex, reversibly inactivated the corticofugal feedback loop, and resulted in the amplitude of the light responses decreasing in the majority of relay cells. Kalil & Chase also found a consistent decrease in spontaneous activity, but the *rebound* discharge (excitation) that followed the inhibitory phase of the light response was *enhanced* by cortical cooling. The last point is

relevant to the origin of the post ED (early discharge) inhibition seen in geniculate cells. i.e. it can be deduced that there must be other paths that control the post ED inhibition. This is because even though the rebound activity is potentiated with corticofugal "blockade", the post ED inhibition remains. If the cooling of cortex blocks almost all of the corticofugal input, then we can ascribe the corticofugal (corticoreticular and/or corticothalamic) input to having excitatory and inhibitory effects on geniculate relay cells. In fact, Widen & Ajmone-Marsan (1960) demonstrated that electrical stimulation of visual cortex had excitatory and inhibitory effects on geniculate cells. In this instance, depending on the depth of the stimulating electrode in cortex, it may be difficult to separate or distinguish antidromic from orthodromic stimulation.

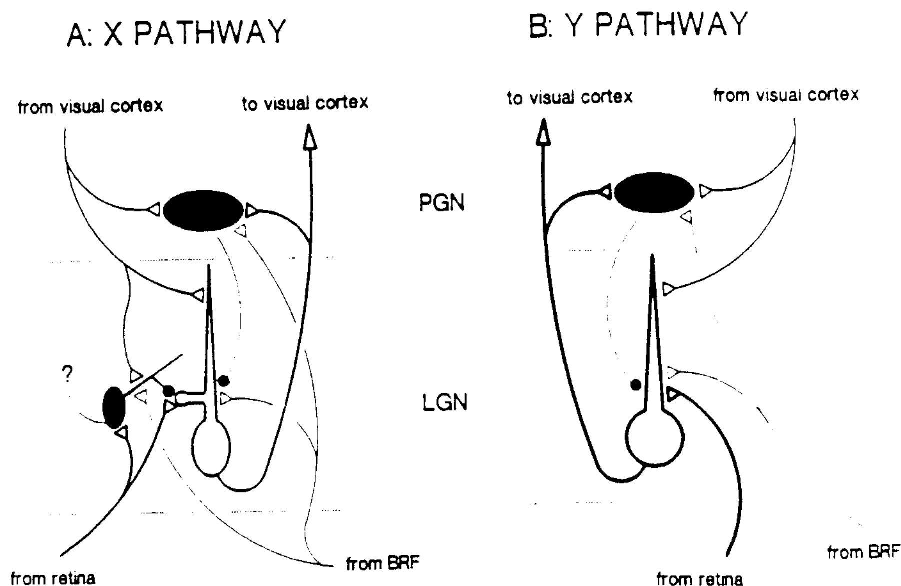


Fig. 2.5. A schematic representation of X and Y circuits for the A-laminae of the cat's LGN. **A.** X pathway. Much of the input to X relay cells (unshaded), including inputs from the retina, from the dendrites of interneurones (filled), from the perigeniculate nucleus (PGN, a part of the RNT), and from the brainstem reticular formation (BRF), is filtered through the glomeruli (stippled region). Retinal terminals engage in triadic relationships with terminals from the interneurone's dendrites and dendritic appendages on the relay cells. The interneurone is also innervated from the retina, cortex, and the BRF; the target of the interneurone's axon remains unknown, except that it is extraglomerular. The PGN cell is innervated from geniculocortical axons, corticogeniculate axons, and BRF axons. **B.** Y pathway. This circuit is much simpler, because there is an apparent absence of interneurones. The retinal axon contacts the relay cell on proximal dendritic shafts among axon terminals, from cortex, PGN, and brain-stem. Cortical and brainstem inputs to relay and PGN cells are similar to that shown in A. Sherman & Koch (1990) claim that some PGN axons can innervate both relay X and Y cells (from Sherman & Koch, 1990).

None of the studies cited in the last paragraph consider the spatial component of the corticofugal input on geniculate relay cells. Further, the cortical cooling experiments are controversial. Some researchers have found that cortical cooling reduces the activity of LGN relay cells while others find no effect (see Singer, 1977 for a review). Perhaps the intensive research effort into possible cortical mediated inhibitory effects on geniculate neurones was based on the fact that there are approximately one order of magnitude more corticothalamic axons than thalamocortical ones, and that each cortical axon innervates many thalamic neurones (Singer, 1977; Sherman & Koch, 1990). Some insight stems from a study by Tsumoto *et al* (1977), cited by Singer (1977). They found that microiontophoretic application of Glutamate (a common excitatory CNS neurotransmitter) in layer 6 visual cortical cells, had an excitatory action on LGN relay cells. However, when the injection site was out of register with respect to the investigated LGN projection column, then the effect of glutamatergic injection was inhibitory on LGN relay cells. Thus, the geniculo-cortico-reticular circuit shown in Fig. 2.6 is probably one module which is (in the intact brain) necessarily interconnected with other similar adjacent modules (circuits).

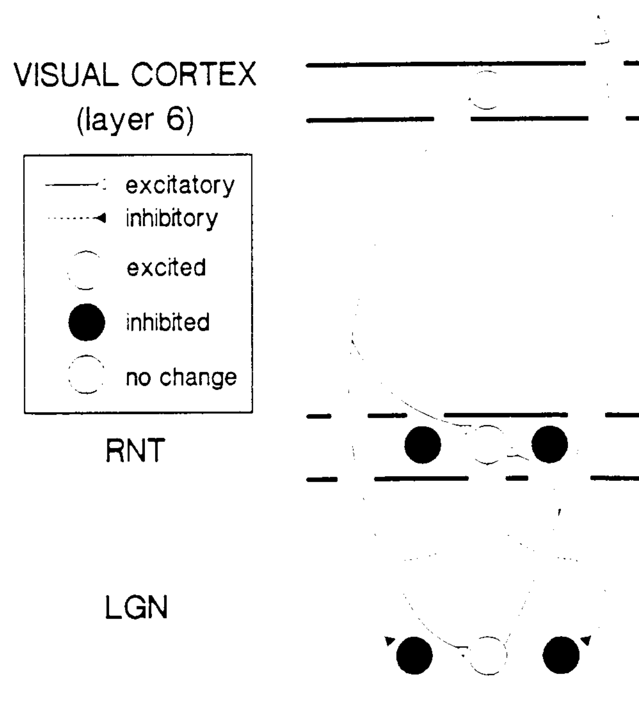


Fig. 2.6. A hypothetical circuit in which the relay cells and the RNT are connected with a lateral displacement. Activity of a relay cell will then disynaptically inhibit its neighbours. Because these neighbours normally activate RNT cells that inhibit the relay cell in question (not shown), this leads to dis-inhibition of that relay cell. Furthermore, a similar displacement of corticothalamic connections will both excite the relay cell and dis-inhibit it (from Sherman & Koch, 1990).

But how does the cortical input mediate both excitation and inhibition in geniculate cells? One possibility would be that a direct corticothalamic input is responsible for potentiated excitation in LGN relay cells that are in register with a cortical cell (i.e. has the same receptive field location and therefore retinotopically mapped with certain cortical cells). At the same time, these cortical cells would have an inhibitory effect on LGN relay cells in adjacent modules. This inhibition would be indirect via the cortico-reticular-geniculate loop (see Fig. 2.6). While this circuit can explain the stronger surround inhibition seen in geniculate cells (as compared to the RGCs'), it fails to explain properties like end-stop inhibition recently seen in LGN relay cells.

Apart from a post-synaptic $GABA_B$ mediated mechanism, only the inclusion of intrinsic inhibitory conductances could explain the protracted (150 ms) inhibitory events in geniculate neurones. In the case of the latter, a calcium mediated potassium ($IK_{Ca}{}^{2+}$) current might be activated and maintained for 100-200 ms. The sequence of events that lead to this type of inhibition is very complex, and has been discussed in detail by Sherman & Koch (1986) and McCormick (1990). They attribute it to the low-threshold (LT) Ca^{2+} current. Briefly, depolarising the neuronal cell membrane above "normal" resting levels (-55 to -60 mV), the LT spike current is completely inhibited (blocked). If the membrane potential is taken slightly below resting membrane potential (e.g. -60 to -65 mV) then the calcium (LT) spike is de-inactivated. *De-inactivated* is used because it now cannot propagate the calcium current by itself but rather requires a small depolarisation (e.g. EPSP). This depolarisation, together with the low threshold calcium current, will activate the $IK_{Ca}{}^{2+}$ (afterhyperpolarising) current that will inhibit the cell for a long duration. Jahnsen & Llinas (1984) have conclusively shown that, at least in brain slices, the long duration post-burst inhibition is mediated predominantly by a calcium dependent K^+ current.

Earlier in this review, it was mentioned that the output of geniculate neurones is limited to the visual cortical areas and the RNT. Based on his electron microscopic studies, Matthews (1973) argued that since the bulk of cells in the LGN degenerate rapidly after visual cortical ablation, the cortical projections appear to be the major output of the LGN. This rapid degeneration of geniculate cells upon visual cortical ablation is consistent with the findings of Sumitomo *et al* (1977). Consequently, if cortical lesioning experiments are to be used in an attempt to remove the post ED inhibition in geniculate relay cells, and therefore determine the extent of corticoreticular inhibition, they should be chemically/pharmacologically mediated and

preferably reversible, instead of the irreversible surgical ablation techniques used by Matthews (1973), Sumitomo *et al* (1977), and Murphy & Sillito (1987).

2.3.3. *Functional significance of the thalamic circuits*

One reason for the LGN being labelled a simple relay station is because it was generally accepted that the response pattern of the relay cells differed only marginally from those in the afferent (retinal) fibres, quite unlike that seen across the geniculocortical pathway. This conclusion may have come not only from electrophysiological evidence but also from anatomical and morphological data. For example, Singer (1977), based solely on the fact that the major excitatory drive of any geniculate cell comes from only one (consistent with Sherman & Koch, 1986, 1990) to a maximum of six retinal ganglion cells, concluded that not only are the functional characteristics of RGCs basically unaltered during thalamic transmission, but also the size of the excitatory receptive field remains comparable. Ironically, Singer (1977) then argued that the LGN is not a simple relay station because of its *complex* connectivity and *mode of operation*. These ideas are consistent with Crick, 1984; Sherman & Koch, 1986; Martin, 1988c; and Sherman & Koch, 1990. Some scientists mention that there is overwhelming evidence to suggest that input to the LGN dominates from areas that are non-retinal (e.g. RNT and cortex). Retinal input to the LGN makes up only 20% of the total number of synapses (Sherman & Koch, 1990). This is somewhat analogous to the cortex, where the total number of geniculate synapses onto any single cortical cell makes up only 20% of the total synaptic input (Douglas & Martin, 1990).

Why is it that even the most recent reviews on geniculocortical function (e.g. Sherman & Koch, 1990) ignore the findings of Murphy & Sillito (as discussed above) and insist that there is a negligible transformation of receptive field structure from the retina to the LGN? Perhaps the receptive field properties of *single* cells cannot be functionally related to the performance of specific microcircuits. This is very much our view today, as will become more apparent in chapter 3 when the cortical microcircuits are discussed.

Singer (1977) said that the most intriguing discovery was that the transmission properties of relay nuclei are controlled to a considerable extent by corticofugal and ascending reticular pathways. Singer (1977) and others (Sherman & Koch, 1990) concluded that the transmission of sensory

information from the sense organs towards the neocortex was strongly dependent on the internal state of the brain and that the thalamic "relay" nuclei were critically involved in such gating functions. It is clear that global (and perhaps long term) gain controls are set by direct and indirect brainstem terminations on the geniculate relay cells. The level of anaesthesia or the animal's state of consciousness does appear to alter the filtering capacity of the RNT (Sherman & Koch, 1990). The issues that are puzzling are exactly how the LGN and the RNT are involved in contributing to visual perceptual tasks e.g. controlling thalamic excitation so that the image can be analysed by the various cortical areas; and also whether an internal searchlight really exists to "*make the hot areas hotter and the cold areas cooler*" as described by Crick (1984).

In concluding this chapter, it is clear that the LGN *together with the RNT* is not a simple relay station, and is at the very least an active filter that selectively modulates the flow of excitation coming from the retina en route to the visual cortex. The discovery of the correct circuits involved with various aspects of visual perception would come only from a rigourous combination of theory, biological experimentation, and computer modelling. Because the real thalamic and cortical circuits are so tightly coupled, I envision that such modelled or artificial visual circuits will necessarily incorporate the relevant thalamic and cortical microcircuitry in symbiosis.

Chapter 3

LITERATURE REVIEW

VISUAL CORTEX

3.1. Introduction

As physiologists, perhaps our ultimate goal would be to understand how the circuitry of the cortex and their respective neuronal populations operate in visual perception e.g. orientation selectivity, binocular vision, motion detection and the coherent perception of an entire image. It is generally accepted that neocortex is involved with "higher level" or "intelligent" behaviour (see chapter 1). In man, the neocortex accounts for 80% of the total brain volume. The uniformity of cortex not only in different areas e.g. somatosensory, motor and visual, but also in different species has been well documented (chapter 1). The recent excitement about neocortex and it's functional operations come from physiologists working in various cortical areas, and those who believe that the neocortex in general, performs the same basic operation everywhere irrespective of whether it is striate, somatosensory or motor cortex.

A group of scientists that produce what is commonly referred to "neural network" or "connectionist" models are also probing the computational properties of the neocortex for a new inspiration to building intelligent artifacts (see Anderson & Rosenfeld, 1988; Crick, 1989; Douglas & Martin, 1990; Zornetzer *et al*, 1990; for reviews). Actually, many of these networks are just elaborations of the perceptron, a simple single-layered pattern recognising network designed in the late 50's by Minsky & Papert (1969). The design of the current neural nets were envisioned in the late 50's and 60's, but they could not be put to practical use because at that time, the classic artificial intelligence (AI) workers had no way of assigning the correct synaptic weighting to the "hidden" layers of these artificial neural nets. Today, the success of "neural networks" stems largely from the development of the "back propagation of errors algorithm" (or backprop) which successfully trains the network by assigning the correct synaptic weightings to the appropriate layers (Sejnowski & Rosenberg, 1988; Crick, 1989). Upon closer analysis however, we find that these artificial neural nets bear just a passing

resemblance to real brain networks, as their structure and mode of operation deviate markedly from the real cortical circuits.

The historical changes in the approach to studying cortical processing since the pioneering work of Hubel & Wiesel has been discussed in chapter 1. The focus of this chapter is to synthesize the relevant microanatomical, electrophysiological and computational data on the visual cortex. Where possible, anatomical and functional differences between the rat and cat visual cortices will be commented on.

3.2. Neuronal composition and basic circuitry of the visual cortex

The most prominent feature of neocortex in general, is its characteristic six layered lamination. This is as a result of differences in packing densities, soma size, the shape of the neurones, and the fibre composition in the respective layers (see Martin, 1988b; White, 1989; Douglas & Martin, 1990). Surprisingly, the visual cortex of the rat is approximately the same thickness as that of the cat and monkey. i.e. 1.5., 1.24, and 1.62 mm for rat, cat, and monkey respectively (Peters *et al*, 1985). Per mm^2 of visual cortex, there are 120 000 neurones in the rat, 60-80 000 neurones in the cat, and 202 000 neurones in the monkey (Peters *et al*, 1985). However, large differences between these species become evident when the total number of neurones (per hemisphere of primary visual cortex) are considered. Thus, there are 1 million neurones in area 17 (primary visual cortex) of the rat, 29 million in the cat, and 160 million in the monkey (Beaulieu & Colonnier, 1983). Because of the similar six layered neocortical structure across different species, "elaborate" computations of "higher" mammals e.g. primates, might simply be as a result of increased numbers of "modules" instead of increasing complexity of synaptic connections within restricted populations of cortical neurones.

To a first approximation, the visual cortex (and neocortex in general) contains just 2 basic types of neurones i.e. cells that have spiny dendrites and those that have smooth dendrites (see Douglas & Martin, 1990). Others, (Peters, 1985; Peters *et al*, 1985; and White, 1989), have opted for a slightly different classification i.e. they have grouped the cortical neurones into pyramidal and non-pyramidal cells. These differences are academic, and for the purposes of this review I will conform to the classification of Douglas & Martin (1990). It is generally accepted that spiny neurones are excitatory whereas smooth neurones are inhibitory (White, 1989; Douglas & Martin, 1990).

3.2.1. *The Spiny neurones*

The spiny neurones are classified into pyramidal and stellate varieties. The pyramidal cells appear to be the major morphological type, with Douglas & Martin (1990) estimating them to account for 66% of the total neuronal population in neocortex. Peters (1985) and Peters *et al* (1985), based on counts in the rat visual system, estimate the pyramidal population to be between 85-90%. Both rat and cat studies suggest that the pyramidal cells are absent in layer 1. Perhaps the hallmark of pyramidal neurones is the presence of a vertically orientated apical dendrite which typically ramifies several layers of cortex, and is therefore well placed to receive input from the multiplicity of axonal fibres that are known to traverse within specific cortical layers (see Fig. 3.1. C & Fig. 3.3). Pyramidal cells typically have one main axon which projects out of the region of cortex in which the parent cell body is situated (White, 1989). This axon "emits" an extensive horizontal collateral system that forms part of the intrinsic cortical circuitry. Douglas & Martin (1990) have added that the pyramidal cells often project to other regions of the brain, and also represent the major source of output from the cortex (Fig. 3.1). In the cat visual cortex, the pyramidal cells can have simple or complex receptive fields (Douglas & Martin, 1990). In the rat visual system, cortical pyramidal cells can have simple, complex, or even hypercomplex receptive fields (Sefton & Dreher, 1985).

Pyramidal cells make only excitatory (asymmetrical, Gray type I) synapses predominantly on other spiny neurones (rat: see Peters, 1985; cat: see Douglas & Martin, 1990). In the visual cortex, each pyramidal neurone is believed to have approximately 6000 spiny boutons. Pyramidal (and spiny stellates) do not receive excitatory (Gray type I) synapses on their somata. Instead, these synapses occur on dendritic spines (75%) and shafts (25%) and probably act via N-methyl-D-aspartate (NMDA), aspartate (ASP), and glutamate (GLU) amino-acid receptors (Douglas & Martin, 1990; Berman, 1991).

The other type of spiny cortical neurone, the stellate cell, is found exclusively in layer 4 of visual cortex (White, 1989; Douglas & Martin, 1990). Stellate cells also have spiny dendrites but they differ from the pyramidal cells in that they do not have an apical dendrite (see Fig. 3.3). Their dendrites are of equal length and radiate out from the soma to produce a "star-like" appearance (Douglas & Martin, 1990). Also, unlike the pyramidal cells, their outputs and inputs are largely local and therefore seldom project to other cortical areas. Douglas and Martin (1990) add that in the cat visual cortex, all stellate cells

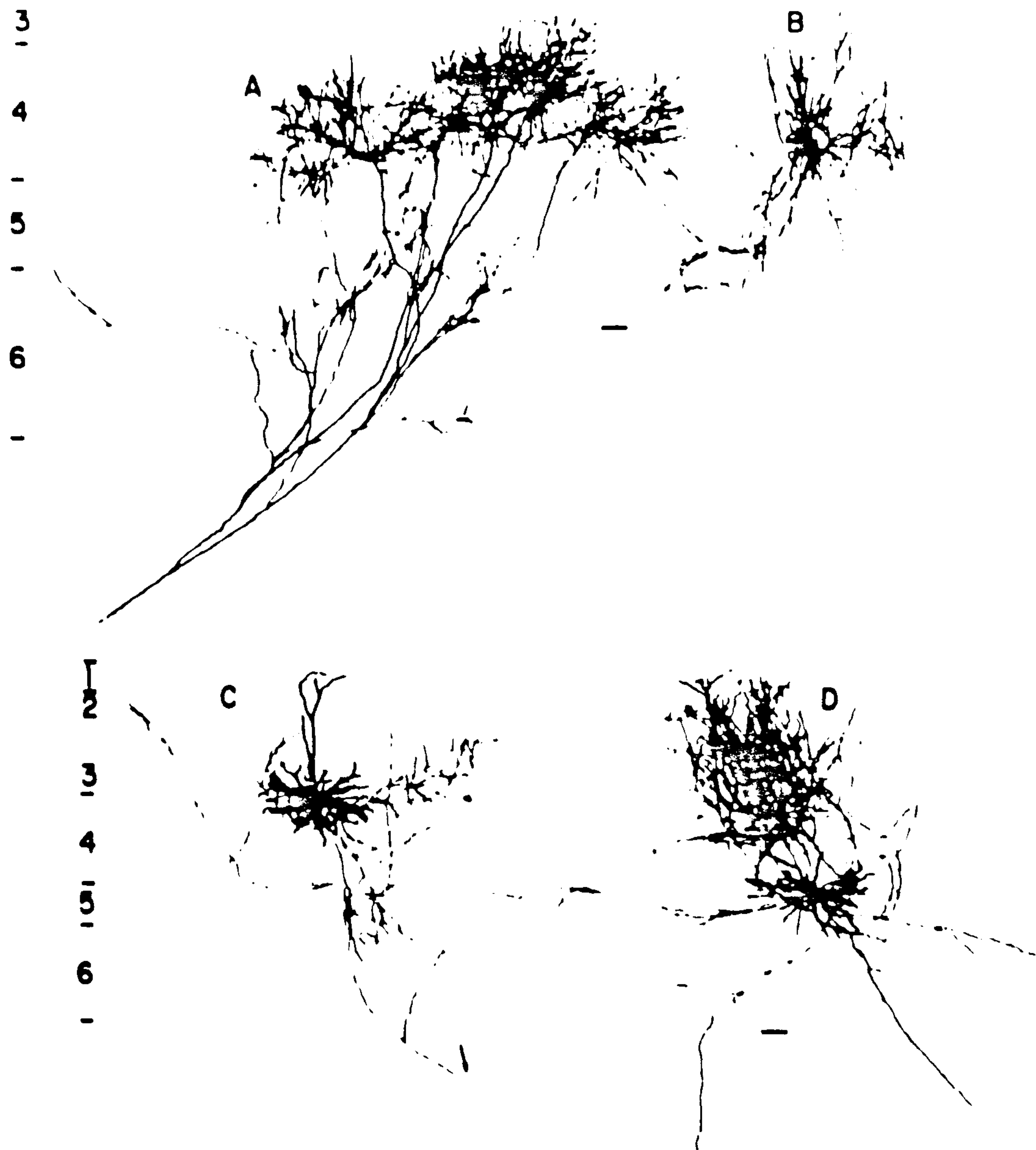


Fig. 3.1. A single thalamic afferent and several spiny neurones from the cat visual cortex that were filled *in vivo* with horseradish peroxidase. **A**. Y-type thalamic afferent. Note the extensive patchy axonal arbours in layer 4. Douglas & Martin calculated that this axon formed over 8000 synaptic boutons. **B**. Stellate neurone (see text for further details). **C**. Pyramidal neurone of layer 3. Note the characteristic apical dendrite extending to layer 1. Many collateral branches arise from the main axon before it leaves visual cortex (arrowed). **D**. Pyramidal neurone of layer 5. This cell type has a very rich collateral axonal arbour in the superficial layers. Cortical layers are as indicated. Bar = 100µm (from Douglas & Martin, 1990).

have simple receptive fields. Their exclusivity to layer 4, together with the fact that they receive a substantial thalamic input may have led to the erroneous assumption that spiny stellates are the nearly exclusive target of thalamocortical afferents (White, 1989). The basic circuit of Douglas & Martin (1990) clearly shows that this is not the case (Fig. 3.3). The location and types of synapses (and their neurotransmitters) on spiny stellates are identical to those on the spiny pyramids. The total number of spines on any stellate cell is approximately 2000, which is a third of that found on pyramidal cells.

3.2.2 *The Smooth neurones*

As their name suggests, these neurones have spine-free dendrites. They constitute a heterogeneous group and are best-described by their axonal arborisations. While at least 10 types of smooth neurones have been characterised, the chandelier or axo-axonic cells (Fig. 3.2 A), the large and small basket cells (Fig. 3.2 B & C), and the "double-bouquet" cells (Fig. 3.2 D) have been found in all cortical areas thus far studied (Douglas & Martin, 1990). In the cat visual cortex, the smooth cells can have simple or complex receptive fields (Douglas & Martin, 1990). In the rat visual cortex, non-pyramidal cells, and therefore presumably smooth cells, have only simple receptive field structures (Sefton & Dreher, 1985). Here we refer to "simple" and "complex" as characterised originally by Hubel & Wiesel (1962).

Smooth cells make Gray type II (symmetrical) synapses with their postsynaptic targets, act via $GABA_A$ and $GABA_B$ receptors and therefore inhibit their post-synaptic targets (Somogyi, 1989, Somogyi & Freund, 1989). Unlike the $GABA_B$ mediated change in K^+ conductance, the $GABA_A$ mediated change in chloride conductance may not be accompanied by a net inhibitory current change. The flow of an inhibitory current will largely depend on the reversal potential of that ion species (in this case, chloride), which will be dominated by the concentration gradient of that ion species across the neuronal cell membrane. For example, if the chloride reversal potential is more positive relative to "resting", then activation of the $GABA_A$ receptors will result in an outward negative current which will depolarise the membrane. Whereas, if the chloride reversal potential is more negative relative to resting then activation of the $GABA_A$ receptors will result in an inward negative current that will hyperpolarise the membrane. Generally however, the chloride (Cl^-) reversal potential is close to the "resting" potential of the neuronal cell membrane, and activation of the $GABA_A$ receptors

produces a large change in Cl^- conductance (gCl) with little change in the membrane potential. It is for this very reason that $GABA_A$ (or Cl^-) mediated inhibition is referred to as silent or shunting inhibition (Berman *et al*, 1992).

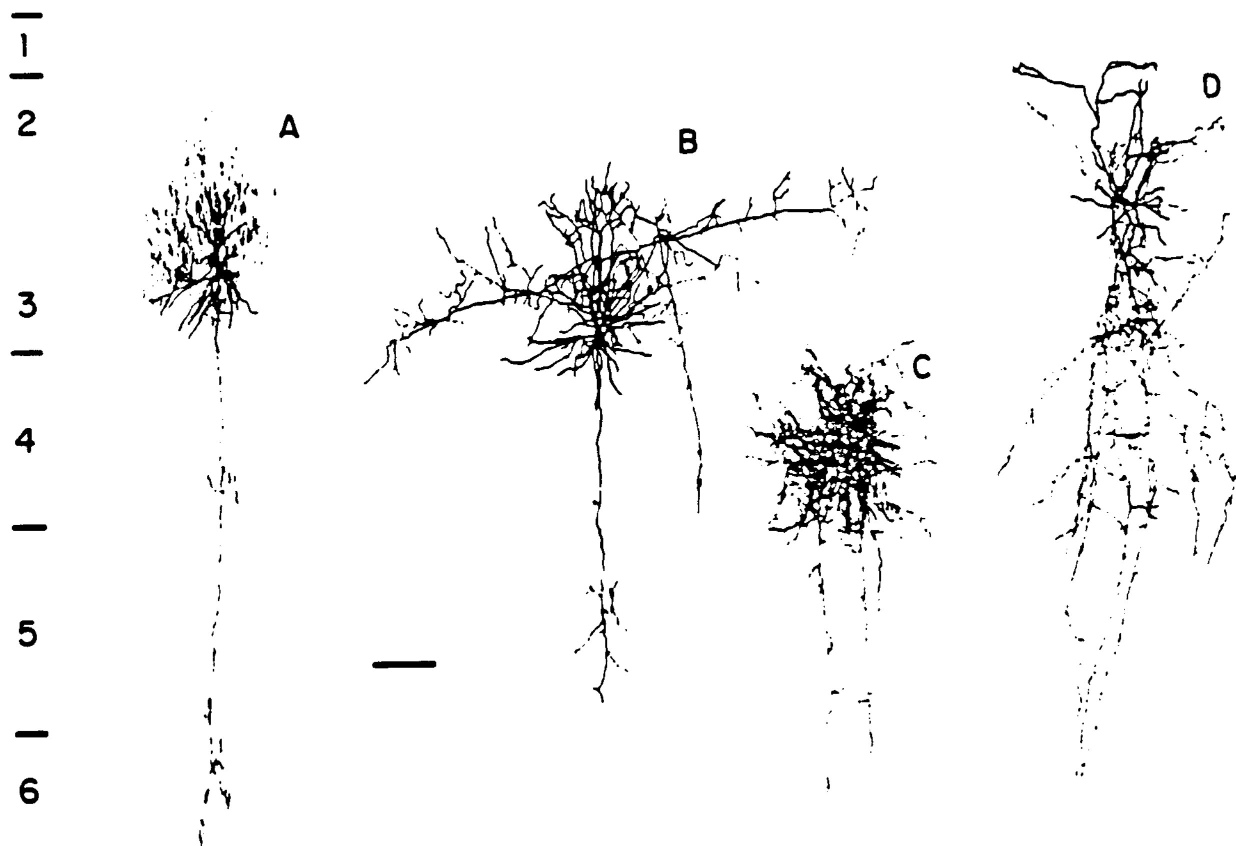


Fig. 3.2. Heterogeneous population of smooth neurones in the cat visual cortex. **A**. Chandelier or axoaxonic cell. **B**. Large basket cell of layer 3. Note the characteristic lateral axon collaterals of this cell type. **C**. Small basket (or clutch) cell of layer 4. In this instance the major portion of the axonal arbour is confined to layer 4. **D**. Double-bouquet cell. The axon collaterals run vertically. Cortical layers are as indicated. Bar = 100μm (from Douglas & Martin, 1990).

Chandelier or Axo-axonic cells

These smooth cells have their synaptic targets only on the initial segment of the axons of pyramidal neurones. Peters (1985) found that in rat visual cortex, chandelier cells are absent in striate (or primary) visual cortex, but are most prominent in the V1-V2 *borders*. No other papers or reviews make such a claim. Nevertheless, the preferential distribution of chandelier axonal terminals on the initial segment of their post-synaptic targets provides these cells with the ability to exert powerful inhibition on the outflow of activity

from the post-synaptic pyramidal neurones. Any single axo-axonic cell provides 5-10 of the total of 40 synapses on the initial segment of any particular pyramidal neurone (Douglas & Martin, 1990). Any single axo-axonic cell nevertheless makes contact with approximately 300 pyramidal neurones. Each pyramidal cell receives an input from 3-5 axo-axonic cells. The superficial cortical layers appear to be innervated with a higher percentage of axo-axonic cells as compared to the deeper layers (Sloper & Powell, 1979; cited by Douglas & Martin, 1990).

Basket cells

Unlike the basket cells of the hippocampus which form synaptic contacts around the somata of their targets, the cortical basket cells synapse mainly onto dendritic spines (White, 1989). It appears as if the basket cells are absent in rats and other "lower" animals (see Peters, 1985; White, 1989). The large basket cells are found mainly in the superficial and deep cortical layers while the smaller basket (or clutch) cells are found primarily in layer 4 and have a characteristically smaller axonal arborisation (Douglas & Martin, 1990). Like the axo-axonic cells described above, the basket cells synapse with approximately 300 target neurones and project predominantly to the layer in which their cell bodies are located (Douglas & Martin, 1990).

Double Bouquet or Vertically orientated cells

As their name suggests, these smooth cells are predominantly vertically orientated, quite unlike the basket cells (White, 1989; Douglas & Martin, 1990). Unlike the axo-axonic and basket cell types, the functional morphology of the double bouquet inhibitory neurone is poorly understood.

3.2.3. *Afferent inputs to the visual cortex*

Thalamus (especially from the LGN)

The geniculate input to the visual cortex has been discussed earlier (see section 2.2.1). Briefly, the primary sites for geniculate terminations are layer 4 and (lower) layer 3 of the primary visual cortex. There are secondary sites on layers 1 and 6. This appears to be the case for rat, cat, and monkey. Note that the somata of those cortical neurones that receive a geniculate input do

not necessarily lie in layer 4, but rather appear to be common in layers 2 through 6. It appears as if all geniculate inputs make only excitatory synapses (Gray type I) with their cortical targets (Peters, 1985; Dehay *et al*, 1991). Unlike the cat, in the rat visual system, there appears to be no segregated geniculate input to striate and extrastriate cortical areas. Douglas & Martin (1990) mention that each geniculate afferent makes only a few synapses with any single cortical neurone. Since the collateral branch of a single arbour has between 1 000 - 10 000 boutons, a single geniculate neurone has the potential to synapse with several thousand cortical neurones. On the other hand, because of the large size of the arbours, any single cortical neurone probably receives input from many geniculate neurones. Immediately apparent is an extensive convergent and divergent "network" across the geniculocortical pathway. It should however be noted that the thalamic input accounts for only 20% of the synapses on visual cortical cells. The remainder originates from intracortical sources (see Fig. 3.3).

BASCIR.DRW

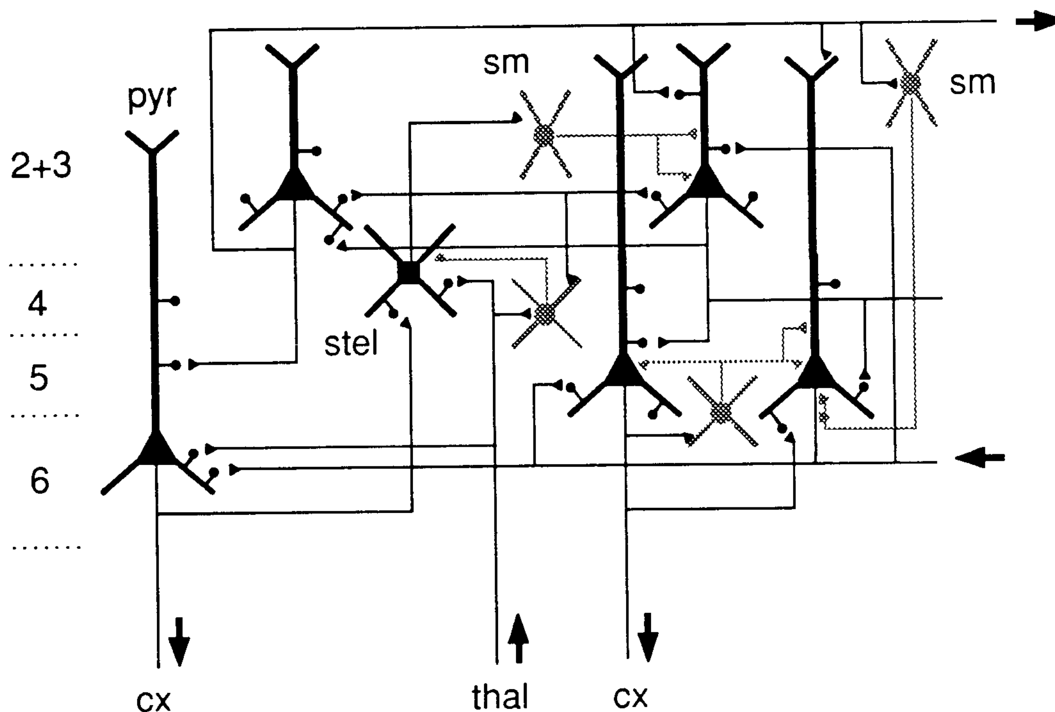


Fig. 3.3. Basic circuit for visual cortex. The spiny (excitatory) cells are filled in black. The smooth (inhibitory) cells are shaded in grey. pyr: pyramidal, stel: stellate, sm: smooth, thal: thalamus, cx: cortex. Note that the synaptic input from the thalamus is small as cortical cells receive most of their synapses from other cortical cells. Cortical layers are as indicated (modified from Douglas & Martin, 1990).

Cortico-cortical connections

The major input to any single cortical area arises from other cortical areas (Douglas & Martin, 1990). While there may be inter-species differences in the pattern of interconnections of different visual cortical areas, it appears as if cortico-cortical connections are made largely by the pyramidal neurones. A general rule of thumb is that the superficial pyramids in one visual area project to other visual cortical areas outside layer 4 (Martin, 1989; personal communication).

3.2.4. *Efferent projections*

The efferent projections to the RNT and LGN were considered in section 2.2.3 above. The pyramidal cells represent the major output source for the visual cortex. Some pyramidal cells from areas 17 and 18 in the rat visual cortex project to the superior colliculus (a nucleus in the brain stem) and the VLG (ventral lateral geniculate nucleus - situated in the thalamus) (Sefton & Dreher, 1985). Further details on these pathways remain absent in the literature.

3.3. Physiology of the Visual Cortex

3.3.1. *Receptive field properties*

The receptive field of a cell in the visual system is defined simply as the region of the visual field that, when stimulated, influences the firing of that cell. Using the Hubel-Wiesel classification, three basic types of neurones exist in the mammalian visual system. i.e. (a). simple cells, (b). complex cells, and (c). hypercomplex cells. The receptive field map of a simple cell usually comprises of elongated ON and OFF regions arranged in parallel. Simple cells are preferentially selective to a specific orientation and position of a stimulus, producing a maximum response in the optimal orientation and/or direction. Complex cells have larger receptive fields with no spatially distinct ON and OFF sub-regions and therefore respond to moving stimuli with tonic (sustained) firing over most of the receptive field (Hubel & Wiesel, 1962; Douglas *et al*, 1991). The hypercomplex cells display "end-stop inhibition" which simply implies that the frequency of the response of that particular cell is reduced as the stimulus is lengthened beyond the optimal length (Mason & Kandel, 1991).

In the cat visual cortex, the majority of neurones have simple receptive fields while in the rat, most of the visual cortical cells have complex receptive fields (White, 1989). However, a significant proportion of rat visual cortical cells display no orientation selectivity, whereas the absence of orientation selectivity in the cat visual cortex is quite uncommon (White, 1989).

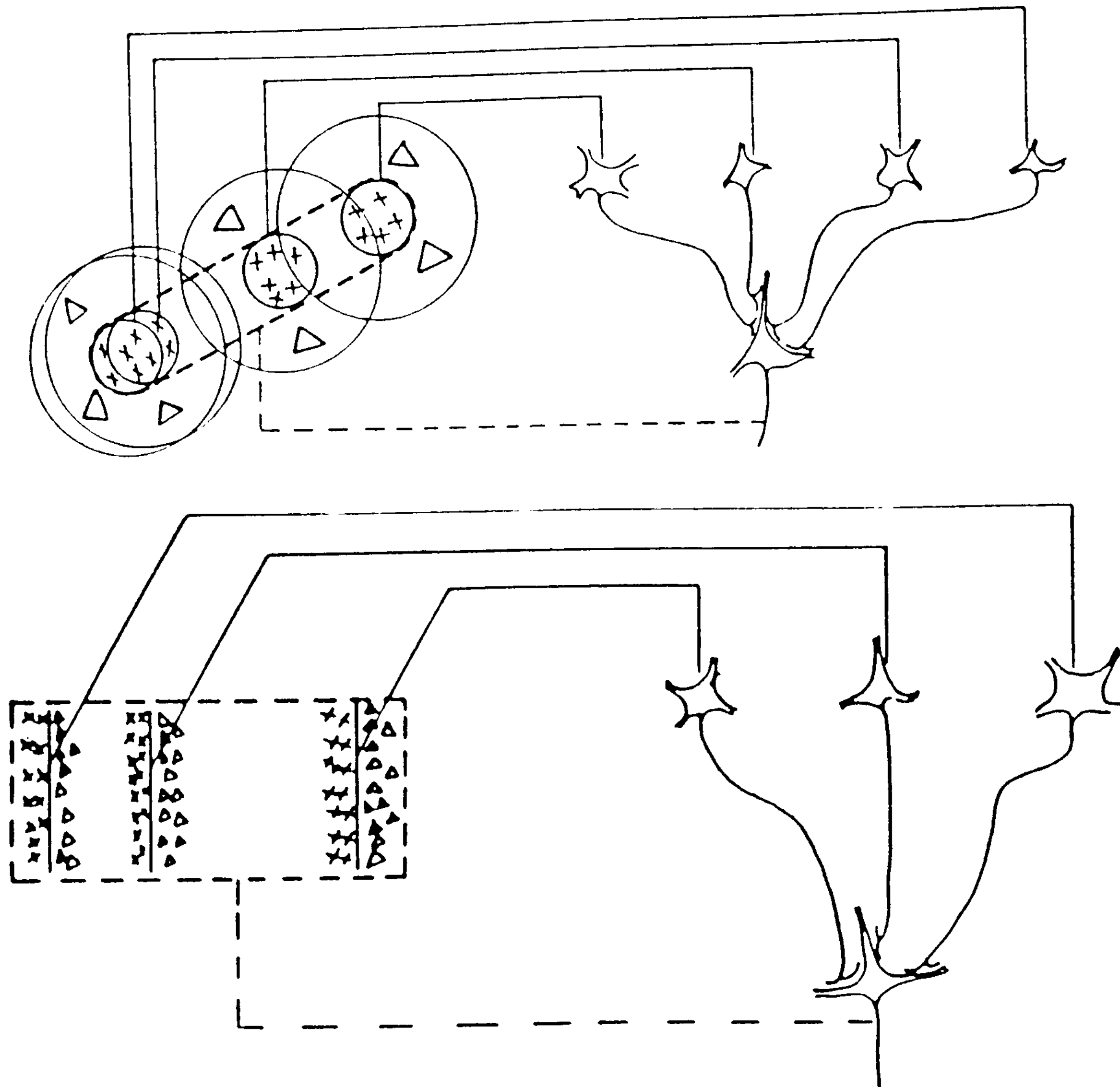


Fig. 3.4. The mechanism for the selectivity of "simple" and "complex" cells as proposed by Hubel & Wiesel. At the top left, the receptive fields of 3 ganglion cells are shown. The messages from a large number of such cells, when lying in a row, are passed through lateral geniculate neurones and converge on a "simple" like cortical cell, which consequently has a linear receptive field. Below are shown the receptive fields of three "simple" cells whose axons converge on a "complex" like cortical cell. Modern techniques have however revealed that individual cortical cells receive few geniculate synapses, and that inhibition may play an important role in orientation selectivity. Also, there is a direct geniculate input to complex cells and not only an indirect one as depicted in this figure (from Hubel & Wiesel, 1962).

The mechanism by which the circular-surround receptive fields of dLGN cells are transformed to the elongated bar-like receptive fields of visual cortical cells is still a matter of heated debate and controversy. Hubel & Wiesel (1962) proposed a hierarchical scheme of arrangement wherein simple cells attained their receptive field structure simply from the convergence of a row of geniculate neurones onto a single cortical cell (Fig. 3.4). The complex cell's receptive field arose from the convergence of many simple cells onto it (see Fig. 3.4). Similarly, the hypercomplex cell's receptive field arose from the convergence of many complex cells. So, according to Hubel & Wiesel, it was clear that the receptive field properties of simple cells were shaped solely by the geniculate inputs, and that of complex and hypercomplex cells were intracortical and hierarchical. Today, there are various lines of evidence that go against Hubel & Wiesel's proposed models of the generation of the various cortical receptive fields. These are discussed below:

Firstly, the Hubel-Wiesel hierarchical model for orientation selectivity of "simple" like cortical cells did not incorporate the role of inhibitory neurones in the visual cortex. Sillito *et al* (1980) have shown that addition of inhibitory antagonists like bicuculline, which compete with GABAergic receptor sites, results in a moderate loss of orientation selectivity in simple cells and complete loss of orientation selectivity in complex cells.

Secondly, it has recently been shown that LGN cells also display end-stop inhibition, a property that was, according to Hubel & Wiesel, reserved only for cortical hypercomplex cells (Murphy & Sillito, 1987; also see section 2.3.1. and 2.3.4.).

Thirdly, some complex (and even hypercomplex) cells are directly excited by geniculocortical afferents i.e. their firing is not dependent on the activation of simple cells (Stone, 1976; cited by Barlow, 1982; Sefton & Dreher, 1985).

Fourthly, it was discovered that some visual patterns that were ineffective for simple cells were effective for complex cells (Hammond & Mackay, 1977; cited by Barlow, 1982).

Fifthly, it is now established that there are extensive parallel pathways within and between visual cortical areas (Martin, 1988b; Martin and Perry, 1988) e.g. the parallel X, Y and W "channels" that are preserved from the retina, through the thalamus and at least until the primary visual cortex (see chapter 2, section 2.2.1). Further, simulations using parallel

processing e.g. neural network models, are considerably faster than those that operate by strict serial processing (Crick, 1989; Churchland & Churchland, 1990).

The five points outlined above clearly go against the proposed hypothetical circuits of Hubel & Wiesel, and forces us to believe that the receptive field properties of single cortical neurones may be shaped by a co-existence of hierarchical and parallel connections. However, they still tell us nothing about the actual microcircuits that are involved in the generation of the various cortical receptive fields. So experimentalists wait to dispel further hypothetical circuits that lack the necessary biological richness. It should be noted that Hubel & Wiesel (nor any other scientists in their era) had no idea of the detailed microanatomy of the neocortex. Today, the cell types and synaptic connections in the visual cortex are well understood, and these have been discussed extensively earlier (see section 3.2). The most obvious microanatomical evidence against Hubel & Wiesel's hypothetical circuits is the fact that the thalamic input contributes just 20% of the total number of synapses on neocortical neurones. Most of the synaptic inputs on spiny and smooth cells (which can have simple or complex receptive field structures) are derived largely from intracortical sources (see Fig. 3.3). Today, we also have electrophysiological evidence that supports this notion that the thalamic (or geniculate) inputs play a minor but nevertheless, essential role in shaping the full-blown response of visual cortical neurones. Part of this evidence will be discussed later in this review.

Douglas & Martin (1990) and Martin (1988a) mention that any single geniculate neurone makes only a few synapses on any particular visual cortical neurone, and that a single LGN neurone cannot drive a "simple" cortical neurone. However, the dramatic divergence of the geniculocortical axon which can make contact with thousands of cortical neurones does not rule out the possibility that any particular cortical neurone receives an (excitatory) input from hundreds of geniculate neurones. Most studies do not consider the preferential weightings of synaptic boutons on any particular cortical cell. Developmental studies suggest that cortical neurones display a high level of plasticity (Martin, 1988a). Consequently, it is possible that the various synaptic inputs onto any cortical cell are weighted preferentially to respond optimally to a specific type of stimulus e.g. a long bright bar. This popular view can now also be finally put to rest because unlike the hippocampus, cortex does not have excitatory inputs from different sources that cluster or form distinct segregations on particular regions of the dendritic tree of individual neocortical neurones (Douglas & Martin, 1990).

3.3.2. *Response to flash stimulation*

Kunt & Creutzfeldt (1971) recorded the intracellular response of cat striate cortical cells to strobe flash (< 1 ms duration) stimulation of the contralateral eye. They reported that the light evoked response consisted of a brief (10-20 ms) initial excitatory (depolarising) phase followed by a longer (50-100 ms) inhibitory (hyperpolarising) phase. Thereafter, there was a sustained excitatory component for at least 150 ms (Fig. 3.5). Kunt & Creutzfeldt focused on the inhibitory post synaptic potential (IPSP) and found that the magnitude of the inhibition was directly proportional to the *intensity* of the flash stimulus. Also, the magnitude and duration of the IPSP decreased with increasing flash frequency, and disappeared at frequencies above 5-10/sec. The decrease (or attenuation) of the early *excitation* at high flash frequencies (above 10-12/sec) was not due to cortically mediated inhibitory actions, but rather because of retinal limitations. This is because Kunt & Creutzfeldt found that the number of spikes in retinal ganglion cells decreased at these higher flash rates.

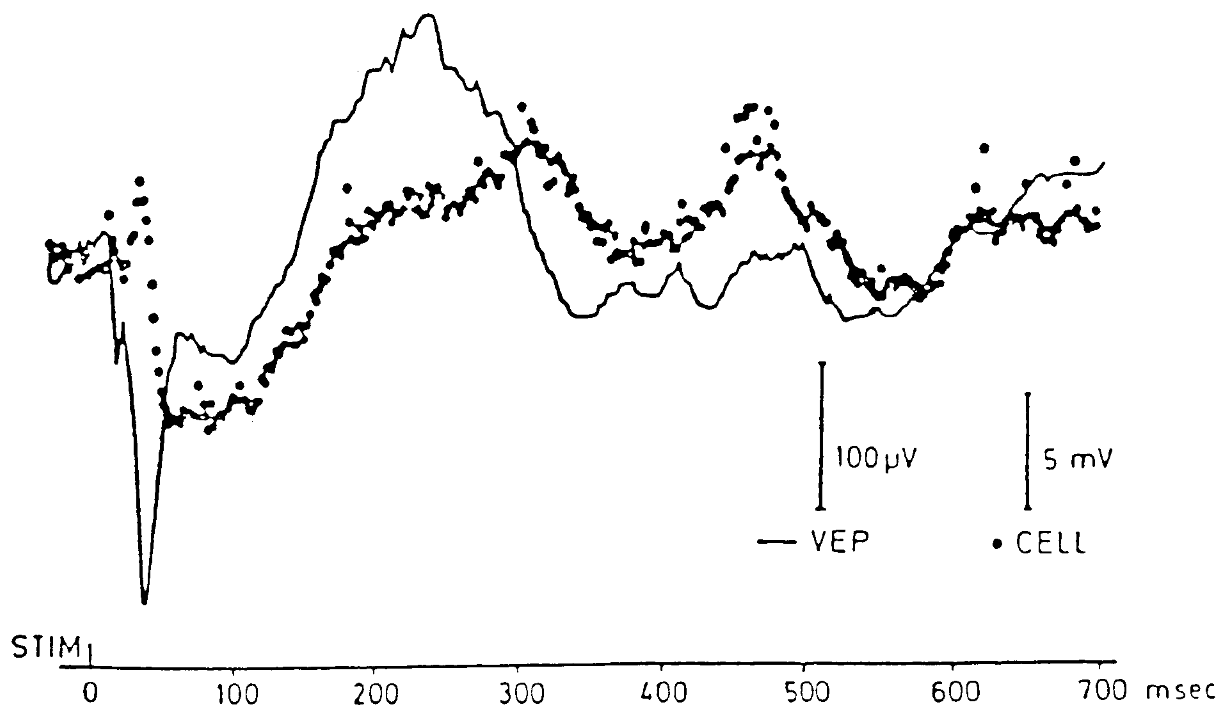


Fig. 3.5. Correlation between intracellularly recorded potential transients and the cortical evoked potential (VEP). Dotted line: averaged intracellular response (30 sweeps, 2 ms analysis interval). Depolarisation in this figure is in the upward direction. Continuous line: VEP (30 sweeps, 2 ms analysis interval. Positive deflection for this EEG electrode is in the downward direction (from Kunt & Creutzfeldt, 1971).

Creutzfeldt *et al* (1969) also analysed the intracellular responses of cat striate cortical cells to strobe flash (10 μs duration) stimuli. They classified their striate cortical neurones into two groups. The first, displayed a primary excitatory phase (30 ms duration) after a latency of 50 ms from the application of the stimulus, followed by either no inhibition, or moderate inhibition. The second group responded with primary inhibition which may or may not have been followed by secondary excitation.

The origin of the late excitatory component of the flash-evoked cortical response in the above studies is critical for any model that accurately predicts cortical processing. This is because such models would need to specify and demonstrate the extent to which these events arise from thalamic and/or intracortical sources.

The motivation for Creutzfeldt *et al's* and Kunt & Creutzfeldt's studies was not to unravel the microcircuitry of cortex and subsequently solve various problems of visual perception. Their goals were focussed on clinical aspects of epilepsy and correlating surface evoked potentials to intracellular responses upon stroboscopic stimulation. They, like Hubel & Wiesel, knew nothing of the detailed cortical microanatomy (Fig. 3.1 & 3.2) and the microcircuits they were embedded in (Fig. 3.3). The reason for including this data here is that Creutzfeldt *et al* and Kunt & Creutzfeldt, even though they probably did not know it, had the right tool i.e. the < 1 ms stimulus, for probing the nature of cortical processing. Recently, Douglas *et al* (1989) & Douglas & Martin (1991) realised the potential gains in using brief pulse stimuli in unravelling the mechanisms of various aspects of cortical processing. This work has made an important contribution to the understanding of cortical processing at the microcircuit level, and will be reviewed in sections 3.3.3 & 3.3.5 below.

3.3.3. *Response to electrical stimulation*

Douglas *et al* (1989) and Douglas & Martin (1991) recorded the intracellular response of cat striate cortical cells to brief (< 1 ms) electrical pulse stimulation of the geniculocortical afferents. They reported that neurones in both superficial and deep layers of striate cortex responded with a short duration (5-30 ms) depolarising potential followed by a long (100-300 ms) hyperpolarising potential (IPSP). More specifically, they found that the pattern of the intracellular response was strongly correlated with the cortical layer from which the neurone was recorded i.e. the initial excitatory phase (EPSP) was larger in neurones that were located in the superficial cortical

layers as compared to those in the deeper layers (see Fig. 3.6). Moreover, the latency to maximum hyperpolarisation of the intracellular cortical response was shorter in the deeper layers (Fig. 3.6). These findings led Douglas & Martin to suggest that stronger inhibition was present in the deeper cortical layers.

Douglas & Martin (1991) also found that the initial excitatory phase (especially for the superficial pyramids) consisted of two separable components. The first excitatory post-synaptic potential (EPSP) was as a result of the thalamic volley of excitation, while the second EPSP was as a result of intracortical re-excitation (Fig. 3.7). Note that this was not a consistent finding in all of their recordings, as in some cortical cells, the thalamic volley of excitation was dominant while in other cortical recordings, the polysynaptic, intracortical excitation dominated the response. Nevertheless, at least for those cell that displayed a clear biphasic excitation (usually coupled with some inhibition), application of bicuculline (a $GABA_A$ antagonist) dramatically potentiated the second phase of excitation without enhancing the initial excitatory phase (Fig. 3.8). As mentioned previously (section 3.2.3), there are no inhibitory (GABAergic) geniculocortical terminations. This suggests that the potentiated bicuculline induced excitation is as a result of *intracortical* dis-inhibition and not as a result of increased geniculocortical excitation.

The classic Hubel-Wiesel model (see section 3.3.1. & Fig. 3.4) that necessarily demanded the convergence of many geniculate cells onto single "simple" like cortical cells, obviously did not incorporate any *intracortical* excitatory (or inhibitory) synaptic connections to account for the final "bounded" receptive field property of these cortical cells. Even though the stimuli used by Douglas *et al* (1989) and Douglas & Martin (1991), were clearly unnatural (probably providing synchronous input to the cortex), their data i.e. the *protracted* coupled excitatory and inhibitory response to brief pulse stimulation, suggests that intracortical processing may play an important part in the moulding of the receptive field structures of visual cortical neurones. The response of striate cortical cells to brief flash stimulation, as that performed by Kunt & Creutzfeldt (1971) (see section 3.3.2), is analogous to the bicuculline induced response to electrical stimulation obtained by Douglas & Martin (1991) i.e. the existence of a prominent intracortical phase of excitation. Perhaps this unihibited response reflects the mechanism by which the population of cortical neurones process visually encoded information arriving from the thalamus.

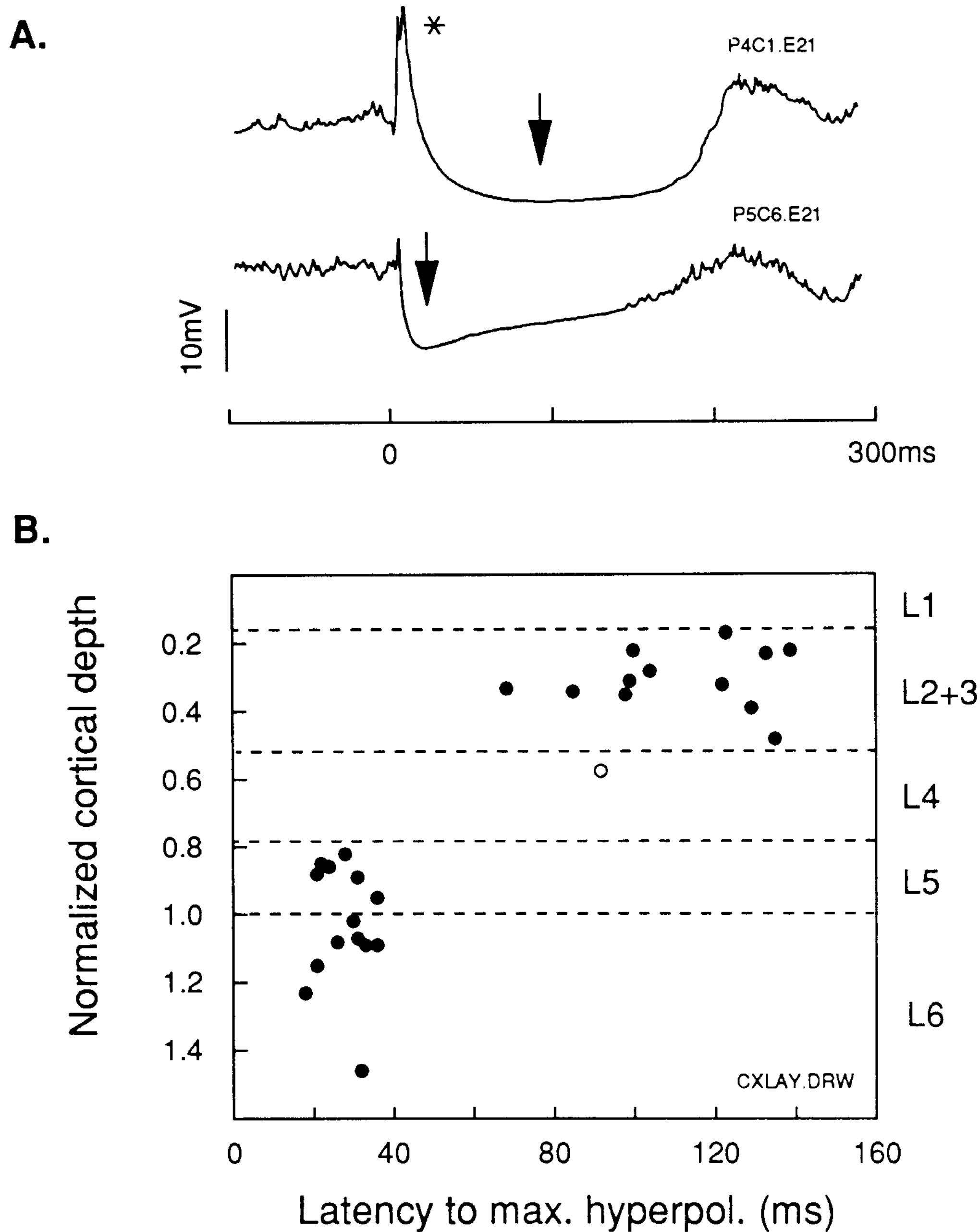


Fig. 3.6. The relationship of the pattern of intracellular responses to stimulation of the geniculocortical afferents with cortical depth. **A**. The hyperpolarising IPSP evolved more slowly in morphologically identified pyramidal neurones of layer 2 and 3 (upper trace) than those located in layers 5 and 6 (lower trace). The latencies to maximum hyperpolarisation (arrowed) were measured with respect to the stimulus at time zero. Superficial pyramids always exhibited marked excitation (asterisk), which was less prevalent in deeper cortical layers. **B**. Relationship between latency to maximum hyperpolarisation and cortical depth for 26 identified pyramidal neurones and one stellate neurone (open circle). Depths of the identified pyramidal cell somata and cortical layer boundaries were measured with respect to the cortical surface and then normalised against the layer 5/6 boundary (adapted from Douglas *et al*, 1989; and Douglas & Martin, 1991).

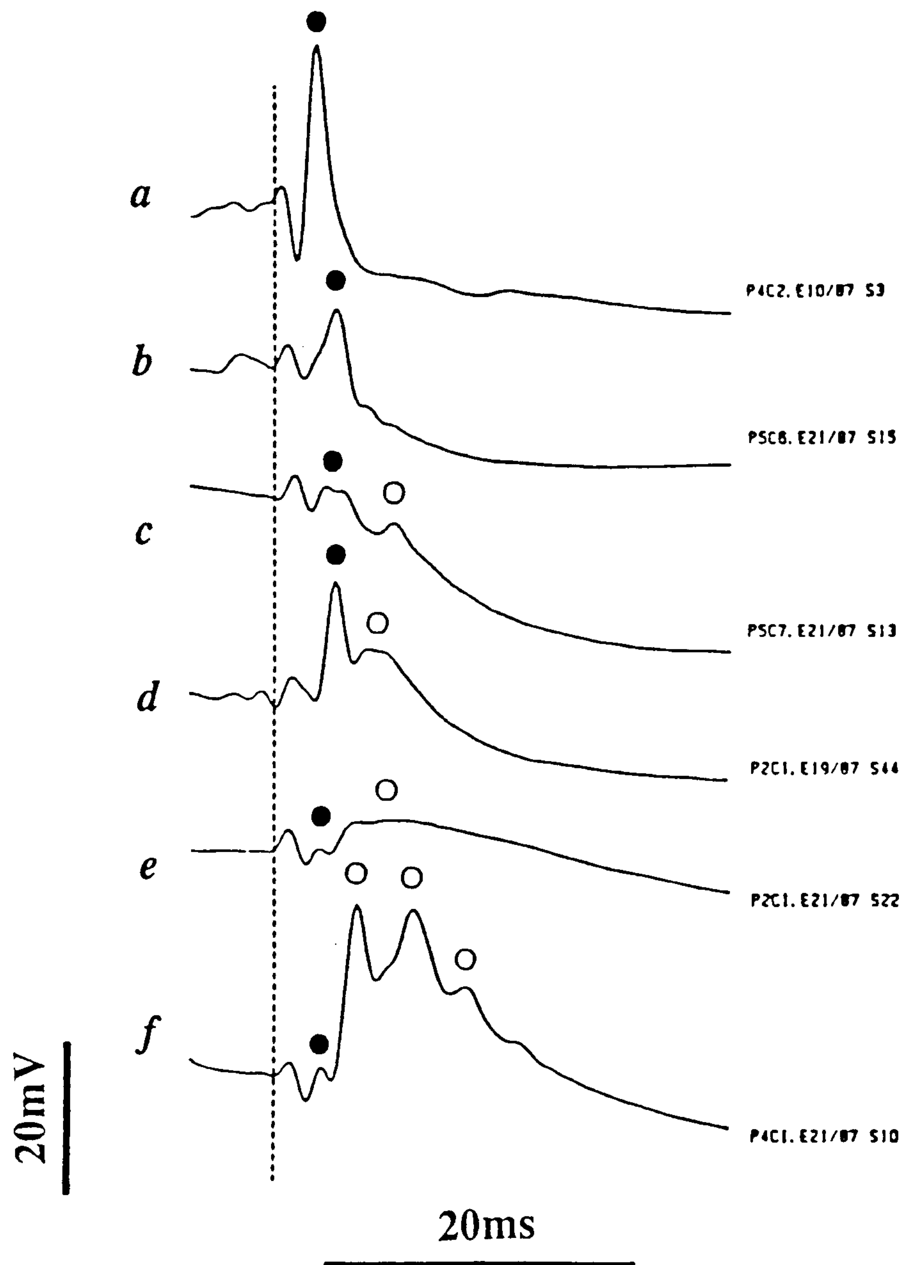


Fig. 3.7. Expanded time views of the early portion of the electrically evoked response of 6 different striate cortical neurones of the cat (**A-F**). According to Douglas & Martin (1990), in most cases, there are at least two separable peaks of depolarisation. The filled circles represent thalamic excitation while the open circles represent polysynaptic (intracortical) excitation. However, this figure shows that some of these "peaks" appear to be below or not significantly different from the control membrane potential [to the left of the dotted line] (see **C** & **E**) (from Douglas & Martin, 1991).

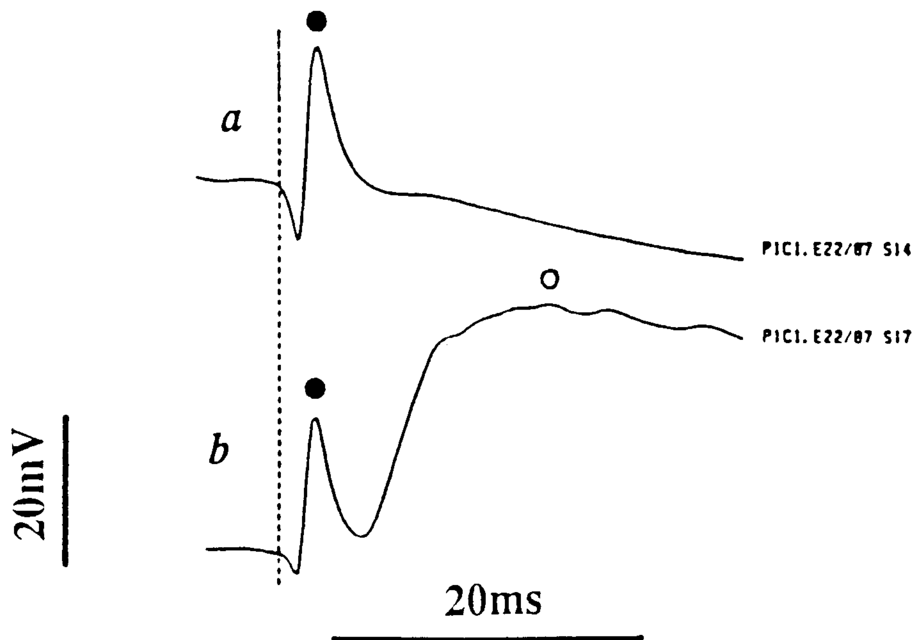


Fig. 3.8. Changes in the electrically evoked response during the application of bicuculline in a single cortical neurone. The first trace (**A**) represents the control. The lower trace (**B**) represents the test i.e. bicuculline application. Note that the amplitude of the early depolarisation, (filled circles) does not increase during application of bicuculline. The late (intracortical) component increases profoundly (open circle) (from Douglas & Martin, 1991).

The remainder of this chapter focuses on neural network and canonical models. Even though they deviate from classic neurophysiology, they have been included in this review because they provide novel insight into certain aspects of cortical processing, that would otherwise not have been possible.

3.3.4. *Neural network models*

For the classical neural doctrine, the activity of a single cortical neurone can be highly significant in causing a percept. For the neural network models however, the activity of individual neurones in cortex is quite insignificant (Douglas & Martin, 1990).

Neural nets are composed of "units" that loosely model *some* properties of the structure of the circuitry of the brain. So, each unit has many excitatory and inhibitory inputs. Each of these will then take the weighted sum of these inputs, and provided that it exceeds some threshold, will produce an output.

An example of such a network is shown in Fig. 3.9 and most commonly consists of just 3 layers. Here, a unit in each of the first two layers connects to all units in the layer immediately above. There are no reverse or side-ways connections in these networks. As already highlighted above (section 3.1), these modern triple layered neural nets by-pass the problems faced by the early networks by using an elegant algorithm (back prop) which will assign the correct synaptic weightings to individual units in the second (hidden) layer until the output appropriately matches the input (Crick, 1989).

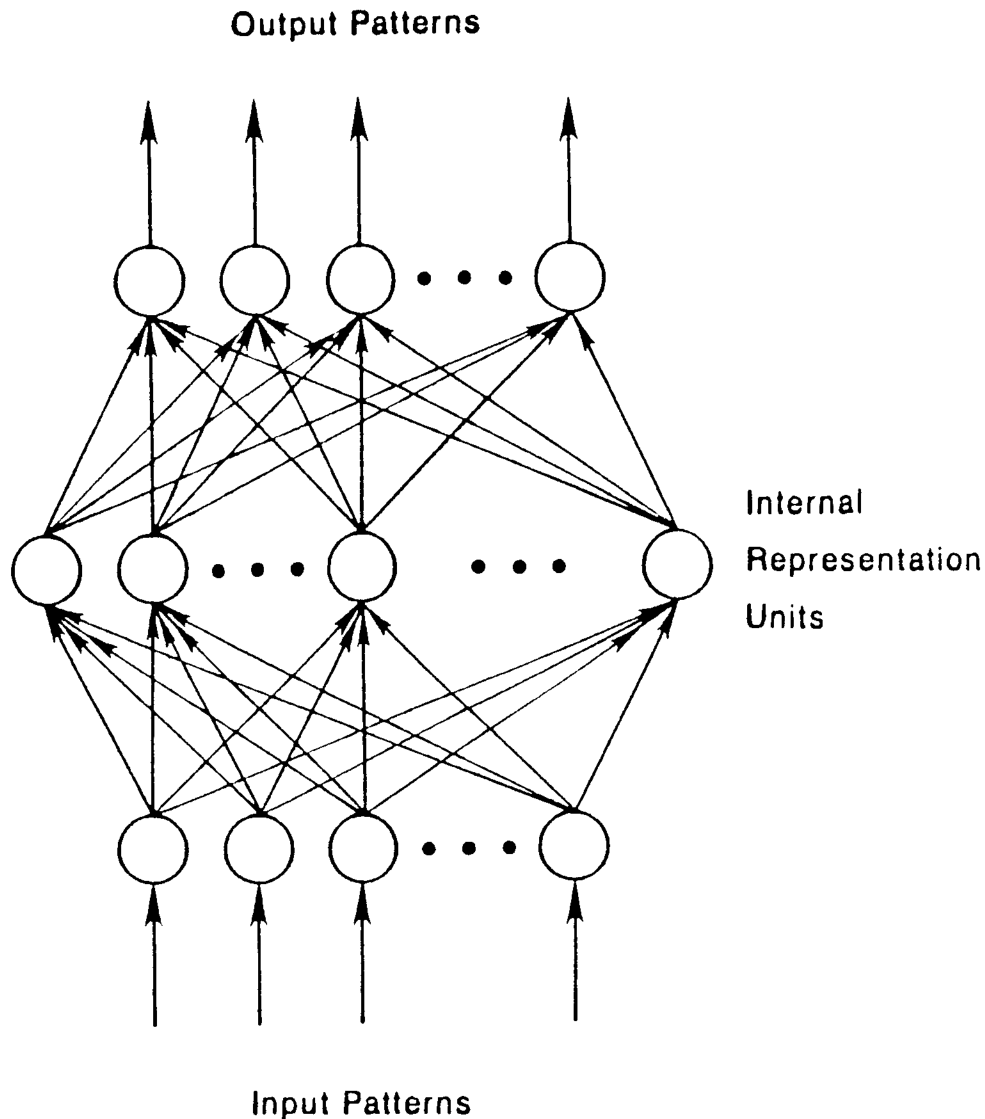


Fig. 3.9. A multilayer network. The internal representation units are sometimes termed hidden units. The information coming to the input units is *recorded* into an internal representation rather than by the original pattern. Input patterns can always be encoded, if there are enough hidden units, in a form such that the appropriate output pattern can be generated from any input pattern. Unlike neocortex, there are no lateral connections between units within a layer (from Crick, 1989).

Sejnowski & Rosenberg's (1988) example of a specific 3 layered network called NETtalk, successfully learned to associate text with phonetic transcription with about 90% accuracy. An important point to mention here is that NETtalk does not perform any better than the classical rule-based models. The advantage of the current network system is that it was easier to construct and handled any language with ease. The competing rule-based systems depended on the painstaking accumulation of decades of linguistic theory, whereas the neural net design simply "learned" from experience. The biologically relevant aspect of NETtalk was the manner in which it learned, making the same over-generalisations that children make in the development of speech. In fact, the neural net even "sounds" like a child learning to speak. Lehky & Sejnowski's (1988) network that derived shape from shading, had a further but more specific message, especially for physiologists working on the visual system. The "receptive fields" of the individual units in the network turned out to be analogous to the edge and line detectors of visual cortical cells. This may imply that the receptive field of single neurones, by themselves, do not necessarily tell us what that neurone's main function is.

The neural network models however, violate some important rules of at least neocortical neurones:

(a). Unlike the "neural" units of networks, outputs of individual cortical neurones are either excitatory or inhibitory, never both.

(b). Neocortical areas have at least twice the number of layers and the extent of the divergence and convergence is far greater than that in the artificial neural network models (Douglas & Martin, 1990). Further, if the back-prop algorithm is generalised to a system of several successive hidden layers, it becomes extremely cumbersome and inefficient.

(c). The real cortical networks have a rich and complicated, but tractable set of interconnections between individual neurones (see Fig. 3.3). The artificial neural nets clearly have a restricted number of connections that can physically be made between the nodes of the network (see Fig. 3.9).

(d). The manner in which the correct weightings of inputs are assigned using back-prop does not appear to be present in the synaptic circuits of the brain as there does not appear to be a *rapid* transmission of information backwards along the axon, and therefore antidromically from each synaptic terminal (Crick, 1989). In all fairness, a similar but much slower mechanism has been *proposed* to occur in the central nervous system (Rauschecker & Singer,

1981). They hypothesized that cortical neurones release a "Synaptic Rewarding Factor" (SRF) when activated and this is picked up by the terminals that have just been depolarised and transported in the retrograde direction to the cell body. This would result in cell growth and increase the strength of the synapse. This is analogous to the way back-prop optimises the correct synaptic weights in the artificial neural nets. The effect of the SRF would presumably evolve over a much longer time scale as it is believed to be related to the plasticity of the visual system during the critical period of development. Such a SRF has yet to be found in the neocortex. For the artificial neural network to be useful, the "teacher" (e.g. back-prop) simply must train the network quickly. If a purely biological based algorithm is sought to "train" the neural nets, then this must necessarily evolve over the time scale of the natural system.

From the preceding discussion, it is clear that the neural network "models" are not really *good* models of the brain because they deviate significantly from the real thing. Their exploitation of parallel processing which dramatically improves the speed of processing as compared to conventional digital computers (Churchland & Churchland, 1990; Douglas & Martin, 1990), together with the back-prop algorithm, might still prove to be very useful in a wide variety of technological and computing applications (e.g. robotics, aircraft surveillance systems, and improving the design of personal computers). From a biological point of view, the greatest and perhaps only attraction of the neural network models is the advantage of parallel processing which appears to be an inherent component of the mammalian brain.

3.3.5. *Canonical model*

We now return to the study of Douglas & Martin (1991) because, apart from their intracellular recordings, they extended their data to develop a canonical model for cortical processing. The neural network models discussed in the immediately preceding section are clearly just gross models of real cortical architectures. The canonical model of Douglas & Martin (1991) however, is biologically based i.e. pays very close attention to the real microanatomy and electrophysiology of neocortex as it arose from the combination of intracellular labelling, intracellular recording, iontophoresis, and circuit modelling using abstract neurones.

Using a general neural network simulating program, the behaviour, morphology and synaptic connectivity of selected populations of neurones were averaged and were each represented by a single compartment. Consequently, the canonical model comprised of three populations of neurones that interacted with one-another i.e. two populations of pyramidal neurones (one superficial layer and one deep layer), and a third population which comprised of (inhibitory) GABAergic neurones (Fig. 3.10). The distinct segregation of the superficial and deep pyramidal populations in the model were based on the difference in the response characteristics of these two populations (see section 3.3.3. & Fig. 3.6).

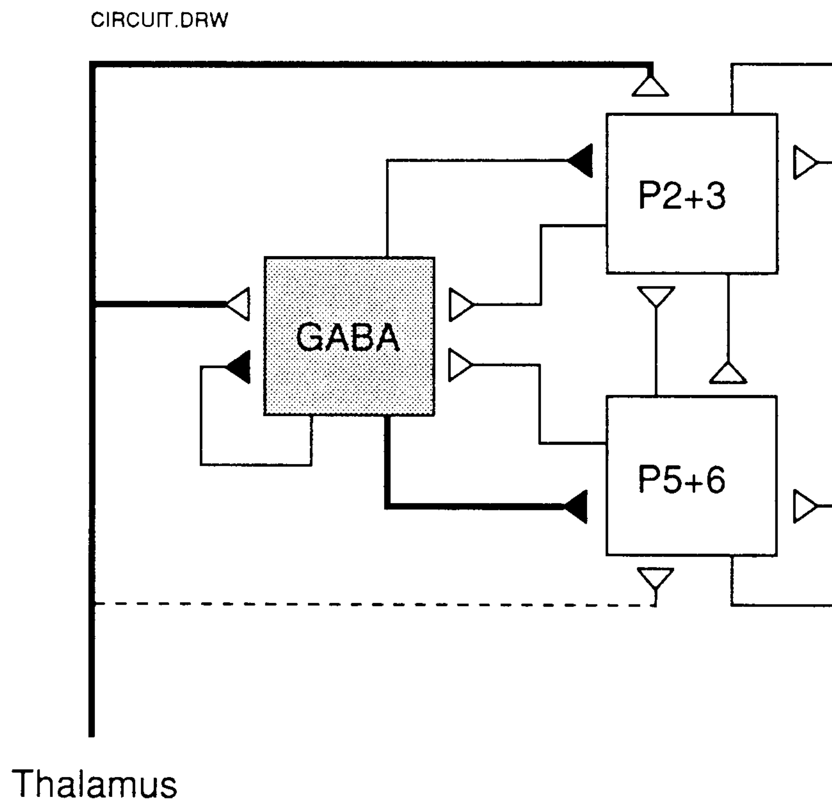


Fig. 3.10. Model of cerebral cortex that successfully predicts the intracellular responses of cortical neurones to electrical stimulation of the thalamo-cortical afferents. Three populations of neurones interact with one another. The first population is inhibitory (GABAergic as depicted by the filled synapses). The other two are excitatory and are represented by open synapses. These comprise of the superficial (P2+3) and deep (P5+6) layer pyramidal neurones. Douglas & Martin have incorporated the layer 4 spiny stellates with the superficial group of pyramids. Each population receives excitatory input from the thalamus , but the thalamic input to the deep pyramidal population is weaker (dashed line). The inhibitory inputs activate both $GABA_A$ and $GABA_B$ receptors on pyramidal cells. The thick line that "connects" the GABA group to P5+6 suggests that the inhibitory input to the deep pyramidal population is greater than that to the superficial population. However, the increased inhibition is due to enhanced $GABA_A$ drive only. The $GABA_B$ inputs to P5+6 are similar to that applied to P2+3 (from Douglas & Martin, 1991).

The *in vivo* intracellular responses of cortical cells to electrical stimulation of the thalamic afferents have already been discussed in considerable detail in section 3.3.3. So we are now in an ideal position to compare those to the simulated responses using the canonical model. Figure 3.11 A shows the response of a superficial and deep pyramidal neurone to electrical pulse stimulation. As shown in Fig. 3.6, the superficial pyramidal cells show a greater degree of excitation than the deep pyramids. Fig. 3.11 B shows the modelled responses for a typical superficial and deep pyramidal cell upon pulse stimulation of the thalamic afferents. Clearly, the modelled responses closely match the *in vivo* responses in many respects i.e. they also show depth related differences in the initial excitatory and subsequent inhibitory phases. To consider yet another example, Fig. 3.12 shows that the effects of pharmacological agents, like bicuculline (a GABAergic antagonist) can also be successfully modelled.

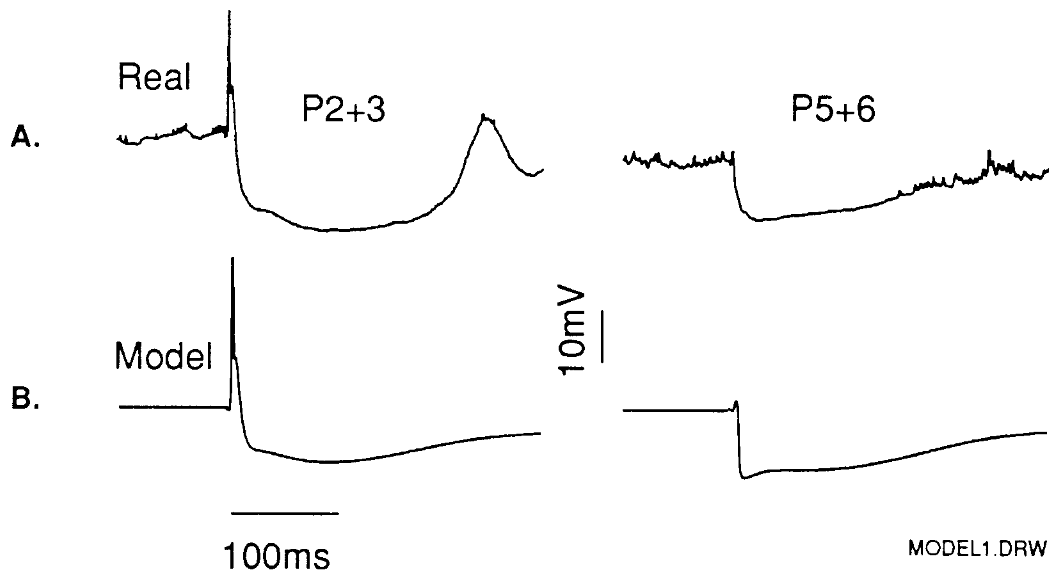


Fig. 3.11. Comparisons of real and model cortical neurones to electrical pulse stimulation. **A.** Response of real superficial (left trace) and deep (right trace) layer neurones. Early depolarisation evoked an action potential in the superficial cortical neurone. No depolarisation was evident in the deep cortical neurone, and hyperpolarisation occurred rapidly. **B.** Model responses of superficial (left trace) and deep (right trace) layer neurones were similar to the real cells (from Douglas & Martin, 1991).

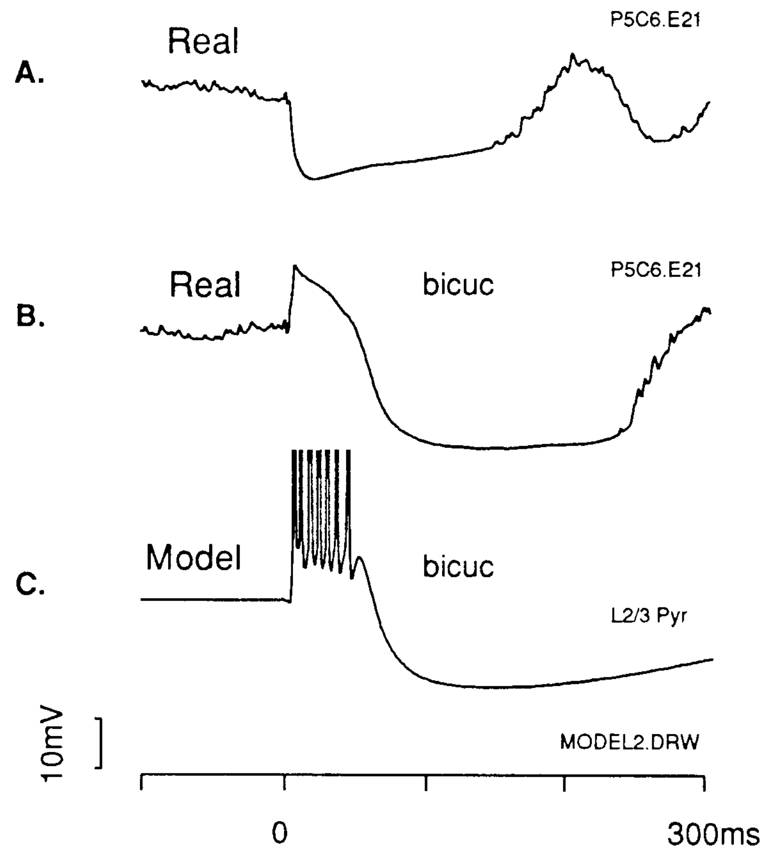


Fig. 3.12. Effect of the $GABA_A$ receptor antagonist bicuculline on the intracellular responses of a real and model cortical neurone to pulse stimulation of the thalamic afferents. **A.** Control response of a real neurone. **B.** Application of bicuculline to the real neurone increased the magnitude and duration of the early depolarisation. Averaging procedure attenuated the action potentials that rode on the crest of the depolarisation. **C.** Simulation of bicuculline application to a model superficial pyramidal neurone gave a similar response to that seen in real neurones (from Douglas & Martin, 1991).

Douglas & Martin (1991) argued that the model is not designed to solve any particular problem in visual processing and is therefore not specific to the visual cortex, but is applicable to all cortical areas. It was earlier mentioned that there appears to be a uniform six-layered structure for all the neocortical areas. This together with the fact that Douglas & Martin (1991) have by-passed the retinal and geniculate input supports their claim for the canonical model being universally applicable to all cortical areas. However, Douglas & Martin (1991) have further extended their model to explain numerous controversies surrounding the generation of various receptive field properties of visual cortical cells. I will not go into the details of these arguments as some of them are very complex and still controversial. The most important prediction of the model however, is that the thalamus serves only to ignite the

cortical circuits, and that intracortical inputs account for most of the excitation seen in these neurones. This is consistent with the overwhelming microanatomical evidence which shows that cortical neurones receive most of their synapses from other cortical neurones, and not from the thalamus. Also, cortical inhibition acts to restrict intracortical re-excitation i.e. inhibition exerts its inhibitory effect by cancelling only small excitatory currents. If the incoming excitation is large, then intracortical inhibition cannot quench it, and so large excitatory signals are always transmitted.

The canonical model may be more relevant to *visual* processing if a segregated and patterned spatial input can be incorporated into it, which will require that the circuit be multi-modular. The other limitation of the canonical model is that it fails to incorporate the fact that there are extensive feedback connections from the cortex back to the thalamus (see chapter 2). Future canonical circuits are thus expected to incorporate the necessary thalamic *and* cortical microcircuitry in symbiosis. The required modifications of the canonical circuit, if it is to mimic cortical processing, will however come only from further *experimental* work and not from mere theoretical considerations. It should be noted that the canonical circuit of Douglas & Martin (Fig. 3.10) takes at least 9 minutes (540 000 ms) to simulate a 400 ms *in vivo* response. The increased time of the modelled response is partly due to the fact that the simulation was performed on a highly serial digital computer (25 MHz 80386/80387 RM NIMBUS VX). The processing time would be significantly shortened if the circuitry of the model was incorporated into an analog electronic device e.g. silicon microchip. Mahowald & Douglas (1991) have taken the first steps in this regard with their silicon neurone. A detailed discussion of the silicon neurone is clearly beyond the scope of this review as it involves the use of complex complementary metal-oxide-semiconductor (CMOS) technology. There is however one very important message that the silicon neurone has for modelers that have been busy simulating various aspects of cortical processing on digital computers i.e. the silicon neurone emulates (and not merely simulates) the performance of real cortical neurones. It operates in real time, consumes very little power, and allows for the integration of many neurones on a single microchip. It clearly supersedes digital simulations on even the fastest of supercomputers. The next steps are that of constructing multineuronal chips that have architectures similar to that of the canonical model.

It is expected that future canonical circuits (digital & analog) will reveal "higher level" cortical function and perhaps allow for the development of truly

intelligent machines. The success of these artificial circuits will however depend on a rich and accurate source of biological data.

In concluding this chapter, we find that until recently, much of the investment in attempting to understand cortical processing proved fruitless. This was because scientists working in this area attempted to provide solutions to explain specific computations like orientation and direction selectivity without an understanding of the basic cortical microcircuits. They paid little attention to the real cortical circuitry and built their theories of cortical processing and various aspects of perception from a tiny window of qualitative electrophysiology i.e. using just the receptive field properties of individual neocortical cells to merely speculate on how the real cortical circuits were synthesizing and processing sensory information.

Chapter 4

GENERAL METHODS

4.1. Animal Preparation, Anaesthesia & Maintenance

Experiments were performed on 74 Long Evans rats weighing between 260 and 350g. All animal experimental methods were officially approved by the University of Cape Town's Animal Research Review Committee.

The animals were anaesthetised with one of the following regimens:
1. i.p. equithesin only (for induction *and* maintenance),
2. induction with halothane (gas) and maintenance with saffan (i.v.),
3. induction (i.p.) and maintenance (i.v.) with equithesin.

After induction, a dual-tubed catheter was inserted into the femoral vein of the deeply anaesthetised rat so as to permit intravenous administration of anaesthetic and paralytic agents during electrophysiological recording. A tracheotomy was performed to facilitate breathing and clearance in the case of any obstruction or mucus build-up. The endotracheal tube also provided a means for mechanically (artificially) ventilating the animal once it was transferred to the stereotaxic apparatus.

Equithesin anaesthesia was used because the anaesthetic is easily administered via the intraperitoneal (i.p.) route, lasts long enough to allow for the completion of surgical procedures, and allows for full recovery of the animal (if so desired). It is also inexpensive and readily available as it can be made-up easily in the laboratory.

The chemical composition of equithesin is as follows:
- 81 ml Sodium Pentobarbitone (60 mg/ml),
- 21 g Chloral Hydrate,
- 10.6 g $MgSO^4$,
- 198 ml Propylene glycol,
- 50 ml Absolute alcohol.

Solution made up to 500 ml with distiled H_2O.
Dose for induction = 0.3-0.45 ml/100gBodyWt, administered i.p.

In those instances where only intraperitoneal administration of equithesin was used for the full duration of our experiments, apart from the initial dose of between 1.2-1.5 ml, supplementary doses of between 0.2-0.4 ml (i.p.) were administered every 45-75 minutes. The interval between maintenance doses depended on the dosage administered and the tolerance of the animal to the anaesthetic. The inherent problem with repeated intraperitoneal injection of anaesthetic agents is that they necessarily result in fluctuating levels of anaesthesia. This makes protracted monitoring of the discharge pattern of single neurones under different test conditions very difficult. Continuous and stable anaesthesia can best be achieved by intravenous administration of the anaesthetic agent. In this respect, continuous intravenous infusion (0.12-0.62 ml/hr) of equithesin proved to be suitable for stable long-term recording of visual units.

Saffan (or alphaxalone-alphadolone), a steroid based drug was documented to be especially suitable for long-term anaesthesia in rats (see Green *et al*, 1978; Green, 1979). Consequently, we explored it's use for our electrophysiological studies. For experiments where surgical and maintenance anaesthesia was achieved with intravenous administration of saffan (12mg/ml), anaesthesia was *induced* with a 2-5% halothane in carbogen (V/V) gas mixture. An adequate level of anaesthesia (during recording) was maintained by continuous intravenous infusion (0.12-0.62 ml/hr) of saffan. Intramuscular (i.m.) injection of saffan had no effect in inducing anaesthesia.

No attempt was made to assess the level (depth) of anaesthesia via classic electroencephalographic (EEG) recordings because the general view today is that it is very complex and unreliable (see Clark & Rosner, 1973; Kulli & Koch, 1991; for reviews). One of the primary reasons for inducing anaesthesia is to block the perception of pain. Consequently, the response to a painful stimulus forms an essential part of assessing the depth of anaesthesia. Absence of the following: pedal reflex, arched back, response to ear pinch, and corneal reflexes, were taken to indicate adequate anaesthesia.

Where paralysis was required to prevent saccades and facilitate mechanical respiration, neuromuscular blockade was obtained by intravenous infusion of pancuronium bromide (0.33mg/ml), administered either continuously (0.2-0.35 ml/hr) or via a bolus injection (0.2-0.4 ml). Paralytic agents were administered only after all surgical procedures were complete, and the animal ready for electrophysiological recording. The required dose of

neuromuscular blocking (and anaesthetic) agents was determined independently for each rat.

Great care was taken to ensure that each and every animal was adequately anaesthetised throughout the recording session. For example, every 20-30 minutes (throughout the 10-18 hr recording session) the level of anaesthesia and paralysis was determined independently and adjusted if necessary. Specifically, the sampling tube used to monitor the level of expired CO_2 (see below) was cleaned every 15-30 minutes to avoid any obstruction of the airways. During this time, we monitored the level of paralysis. In some cases, the animals were breathing spontaneously, indicating that they were not completely paralysed. Irrespective of whether the animal was breathing spontaneously or not, the infusion of the paralytic agent was stopped and we then assessed the level of anaesthesia. In those cases where the animals were not breathing spontaneously (indicating paralysis), within a few minutes, the animals would recommence spontaneous respiration. This range of response suggested that our routine dose of paralytic agent as appropriate. Once the animals started to breathe spontaneously, we observed whether they were experiencing any pain from inadequate anaesthesia. At no stage of the recording session was the infusion of anaesthetics discontinued. We found that as long as the anaesthetics were infused continuously via the intravenous route (with the doses cited above - see page 50) [as they always were in all recordings using paralytic agents], in no cases did the animals show any discomfort whatsoever (e.g. pedal reflexes, arched back, corneal reflexes). It is possible that the animals might have experienced some pain if *surgical* procedures had been performed with doses of anaesthetics used for electrophysiological recording. But this was never done i.e. surgical procedures were *never* performed during *electrophysiological* recording sessions.

Under no circumstances did we administer paralytic agents with intraperitoneal anaesthesia. This was simply because with intraperitoneal administration of any kind of anaesthetic, the diffusion rate of the anaesthetic from the peritoneum into the blood-stream will be variable depending on where the fluid was injected. Consequently, it is unacceptable to administer neuromuscular blockers if the anaesthetic is being administered intraperitoneally because the experimenter cannot ensure that an adequate level of anaesthesia will persist for some protracted duration. On the other hand, use of a suitable intravenous anaesthetic like equithesin or saffan (as done in this study) ensured that a constant supply of that

anaesthetic agent is present in the blood. If for any reason, the animal starts to develop some resistance to that anaesthetic dose, then the dose of the anaesthetic can be increased appropriately in our regular 20-30 minute assessments of the level of anaesthesia (as described above). This was seldom necessary and we often found that the required dose of anaesthetic actually decreased slightly as the experiment progressed.

Extensive pilot studies using the above-mentioned anaesthetics in the absence of neuromuscular blockers were done in the same laboratory during my Honours project (1990) and before the commencement of the Masters project in 1991. This ensured that we understood the kinetics of these anaesthetic agents. Nevertheless, we always continuously (i.e. every 20-30 minutes) assessed the level of anaesthesia for each of the animals used in this project to ensure that they were not experiencing pain in any phase of the experimental procedures (be it surgery or electrophysiological recording). Expired CO_2 was monitored continuously via a sampling tube connected to the endotracheal tube and fed to a Beckman (LB-1) CO_2 analyser. The animals were artificially ventilated and the stroke volume appropriately adjusted so that the expired CO_2 level was kept around 2.8-4%. This range of expired CO_2 levels is consistent with the studies of Kunt & Creutzfeldt (1971) and Gray *et al* (1990) for cats, and Simons & Carvell (1989) for rats. Absence of spontaneously discharging visually driven units was associated with unacceptably low expired CO_2 values i.e. $< 2.0\%$. Any data obtained from these animals were discarded.

The body core temperature was monitored with an intra-rectal thermistor probe and maintained at 38 ± 0.5°C with an electrical heating pad in contact with the ventral surface of the body. Adequate anaesthetic stability and recording conditions generally persisted for 10-18 hours.

4.2. Stereotaxic Procedures

The deeply anaesthetised animal was positioned in a custom-built stereotaxic apparatus. Once the animal's head was securely placed in the stereotaxic frame in the flat skull position i.e. incisor bar 3.3 mm below horizontal zero (as specified by Paxinos & Watson, 1986), the hair covering the cranium was clipped short. A longitudinal midline incision was made through the skin from slightly behind the eyes to the cervical region. A sharp scalpel blade and forceps were used to laterally deflect the skin and remove the overlying connective tissue, fascia and blood vessels. The deflected skin was kept in

place (away from the dorsal cranial surface) by using bulldog clips and artery clamps. The cranial surface was continuously swabbed and the above procedure repeated until landmarks like bregma, the lambdoid and midline sutures were clearly visible.

Depending on which target area was to be investigated (i.e. LGN or striate cortex, or both), a burrhole of at least 5 mm diameter was drilled through the skull using a SS White drill bit (2.2 mm diameter) attached to a Dremel Moto-Tool. This was done under continuous magnified vision using a Carl Zeiss (62108) dissecting microscope. When the underlying blood vessels and dura were visible through the final layer of bone, a fine probe was used to asses the remaining thickness of bone. From here on, the drill bit was changed to a 1.8 mm diameter bit and the final layer of bone was carefully trimmed away. Using a 26G needle, the dura was teased until a tear was made, and was then deflected using a pair of fine forceps.

Bregma was used as the reference point for all cortical and geniculate target coordinates (lateral and anterior-posterior). Using the coordinates of Paxinos & Watson (1986), geniculate stimulating or recording electrodes were always positioned between 3.4 - 3.6 mm lateral midline, and between 3.8 - 4.3 mm posterior Bregma. See sections 4.5, 4.7, & 5.3 also. For recordings in the visual cortex, co-ordinates based on the atlases of Paxinos & Watson (1986) and Espinoza & Thomas (1983) were used. See section 5.2 for further details.

4.3. Electrophysiological Recording and Data Acquisition

Standard extracellular recording techniques were employed. All recordings were made via Clark GC100F-10 (outer diameter: 1 mm) single-barreled glass fibre-filled microelectrodes. These were pulled on a customised vertical pipette puller and subsequently back-filled with 2M NaCl. Under a dissecting light microscope, the tip of the microelectrode was "broken-back" with a scalpel blade to a diameter of approximately 0.5-2μm. For extracellular recordings, acceptable tip impedances were 5-20 MΩ. Considering that the diameters of the cell bodies of geniculate relay cells and striate cortical neurones are in the order of 10-20μm, the above-mentioned recording electrode tip diameters optimised single-unit sampling. A silver wire was placed in the electrolyte solution of the recording micropipette so as to establish electrical contact with the probe of the headstage preamplifier (Neurolog NL 102).

The microelectrode was mounted in an electrode holder and controlled by a hydraulic microdrive (Narishige Model) that had a vertical displacement resolution of 2μm. After positioning the electrode at the appropriate anterior-posterior and lateral co-ordinates, the electrode was advanced smoothly through the striate cortical layers to a maximum depth that corresponded to the underlying white matter (~1.6-1.8 mm from the cortical surface). For the recording of cortical neurones, electrodes were advanced up to a maximum of 1.4 mm from the dorsal cortical surface. For the recording of geniculate units, the electrodes were advanced through the various cortical layers, through the hippocampus, and into the dorsal lateral geniculate nucleus (dLGN) of the thalamus. For all cortical recordings, because of the large burr and the removal of the dura, a 5% agar-saline gel was poured over the opening to minimise brain pulsation.

The extracellular (field) potentials were first led to a high input impedance ($10^{11}\Omega$) DC preamplifier (Neurolog NL 102). The signals were then bandpass filtered (100Hz - 20kHz) and fed through a high-gain amplifier (Tektronix AM 502 differential amplifier), monitored via an audio-monitor speaker system, and also by a continuous (on-line) display on a dual-beam oscilloscope (Iwatsu SS 5702 or Telequipment D1011). The signals were led to a discriminator unit (Frederick Haer), which transformed just the action potentials of the units under investigation into a series of standardised 0.5 ms logic pulses. These output pulses were displayed on a second channel of the dual beam oscilloscope to ensure that the appropriate action potentials were being detected by the discriminator. Using a CED 1401 (Cambridge Electronic Design) intelligent interfacing system and neurophysiological data logging software (SPIKE2), these logic pulses were led (on-line) to a 25 MHz 80386/80387 IBM clone. The 386 machine compiled peri-stimulus histograms and raster plots (in real-time). The bin width, sweep number, and stimulus frequency were set as desired. A schematic representation of the above-mentioned circuit is displayed in Fig. 4.1.

All extracellular recordings consisted of multiple trials. Typically, 40 to 80 trials per test condition. Each trial consisted of a control (100 ms) and a test (400-800 ms) period. The inter-trial interval was 4-6 s. Often, each cell was tested under different conditions e.g. different surround illumination and/or different stimuli e.g. flash vs electrical. Consequently, the holding time of each unit often exceeded 30-45 minutes. A few thalamic and cortical cells were held for as long as 2.5 hrs. This was only possible with continuous and stable anaesthesia. All raw data was stored for further analysis.

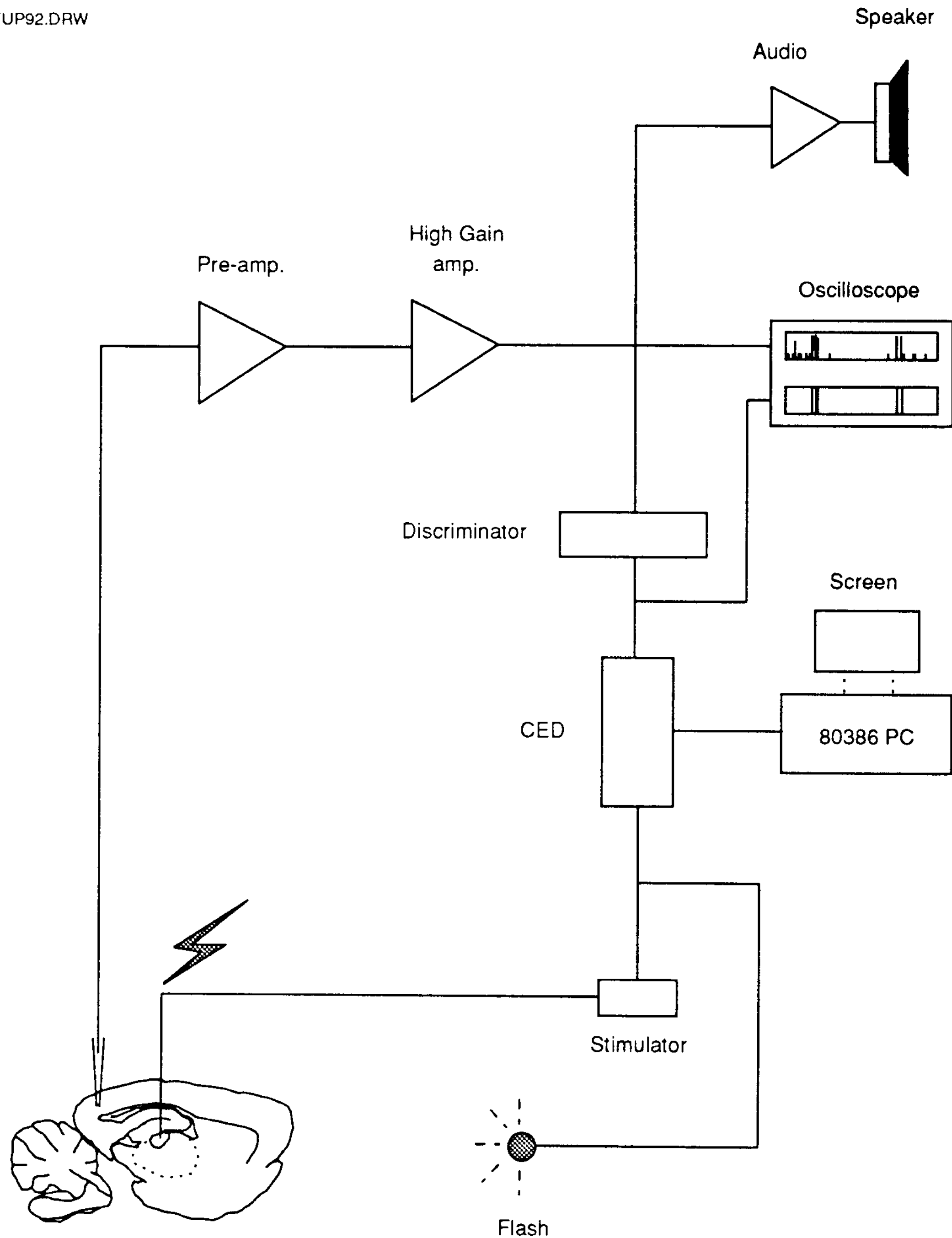


Fig. 4.1. Schematic representation of the electronic circuitry used to record the extracellular response of cortical and geniculate neurones to electrical and/or flash stimulation. Stationary strobe flashes (from a Beckman Photo Stimulator) were triggered via a Cambridge Electronic Design (CED) intelligent interfacing system controlled by customised SPIKE2 software. Monopolar or bipolar electrodes were used to deliver electrical pulses via a stimulus isolator unit (Neurolog NL800), also connected to the CED. All extracellular signals were processed via a headstage preamplifier, filtered and then led to a high gain amplifier, and subsequently monitored on an dual beam oscilloscope.

For the recording of extracellular action potentials, only single units with a signal to noise ratio of greater than 3:1 were considered. Further, it was required that the single units were sufficiently isolated from other surrounding units (neurones). The last pre-requisite for the recording of single neuronal activity was that the spike amplitudes were stable (usually 1 to 10 mV). The electrode was carefully positioned so as to optimise the signal to noise ratio and single unit isolation.

The shape of the extracellular action potentials was not an important consideration in this study since all subsequent data analyses were concerned only with the action potential discharge patterns of thalamic and cortical neurones, as measured from the logic pulses derived from discriminator level crossings (see above). However, the shapes of the action potentials were carefully monitored and noted during data capture to ensure that (a) that the signals were characteristic of a somadendritic rather than an axonal source (see Lemon, 1984 for a review); and (b) that the shape of action potentials being transformed into logic pulses remained constant during the observation period, as confirmation of the validity of the data being recorded.

When a suitable striate cortical or geniculate unit was isolated for recording, it was first allowed to stabilise for 2-5 minutes. If the neurone appeared to be stable by this stage then the receptive field location (and sometimes the receptive field shape) was assessed and noted. After the receptive field assessment, the cortical or geniculate cells were stimulated artificially with electrical pulses and/or naturally with stationary strobe flashes positioned in the appropriate receptive fields.

The data from some animals were rejected because of intolerance to specific anaesthetic regimens, excessive bleeding during surgery, respiratory distress, or incorrect placement of recording and/or stimulating electrodes. No attempt was made to quantify this discarded "data". In a few instances, the animals died before electrophysiological measurements commenced.

4.4. Flash Stimulation Technique

Impulses of light were generated by a Beckman 5561 Photo Stimulator. The delay, interval between successive flash stimuli, and sweep times were controlled by software. The flash unit which emitted 0.05 ms (see Fig. 4.2) "white" strobe flashes, was placed between 0.75 and 1.5 m from the eye of the

experimental animal in the centre of the visual field. The stimuli were always monocular. The non-experimental eye (usually the left eye) was always kept closed. The strobe flashes were flashed directly into the intact eye. The temporal receptive fields were the most practicable ones to stimulate as they receive the largest representation in the retina. Since 95% of the fibres from the rat retinal ganglion cells project to the contralateral LGN and cortex (Sefton & Dreher, 1985), recording and/or stimulating electrodes were always placed in the LGN and/or cortex that was contralateral to the stimulated eye.

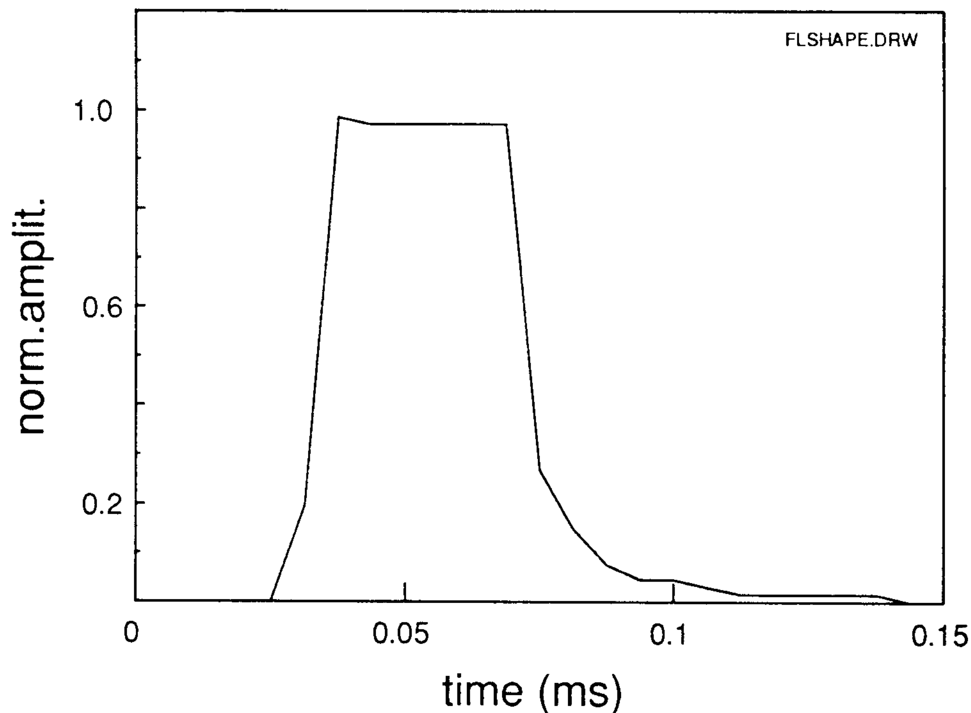


Fig. 4.2. Profile of flash stimulus. Using a fast photocell, the duration of the strobe-flash was found to be approximately 0.05 ms. The shape of the stimulus is to a good approximation, a pulse.

Adaptation luminance was measured with a Hagner Ecies photometer corrected for the human response (*V* - lambda) curve. The unit for adaptation luminance (or constant background illumination) was the lux. The photometer was placed in a visual field and angle similar to that which the subject was exposed to via the strobe flash unit. The intensity of the strobe flash was estimated to be 88 lux (measured at 0.7 m from the subject's eye). The level of constant background (or surround) illumination of the experimental laboratory was always tightly controlled. This was because an earlier study i.e. Creutzfeldt *et al* (1969) found that the temporal profile of the

visual response of some cortical cells could be modulated by small changes in the level of the background illumination (see chapter 3, section 3.3.2 for further details). In our study, background illuminations were kept constant for each test condition. The flash response of individual cortical and thalamic cells were recorded under several test conditions i.e. several levels of background illuminations (0, 1.5, 5, & 90 lux). This ensured that the visually responsive units were logged at the most optimal level of surround illumination.

4.5. Electrical Stimulation Technique

For electrical stimulation of the LGN, custom-built monopolar or bipolar tungsten electrodes (diameter: 0.125 mm) were used. The custom-built electrodes were insulated with epoxylite resin (Clark Electromedical Instruments). The monopolar electrode was supported in a thin glass micropipette and attached to a manual microdrive. Bipolar electrodes were cemented 1 mm apart on a custom-built perspex holder which was attached to a manual micromanipulator. The tips of the stimulating electrodes were exposed using a pair of sharp side-cutters.

Monopolar electrical stimulation pulses were always cathodal (negative with respect to ground). The polarity of the bipolar electrodes were switched during each recording, and the polarity that displayed the smallest artefact and lowest threshold was used. Electrical stimuli were delivered by a stimulus isolator unit (Neurolog NL800), also controlled by customised SPIKE2 software. Electrical pulse stimuli were always 0.2 ms in duration. Multi-unit visually-evoked potentials were recorded from the stimulating electrodes to ensure their correct placement in the dLGN.

4.6. Data Analysis

In addition to the peristimulus time histograms (PSTHs), simultaneous raster plots were also constructed (on-line) to directly observe the stability of each discharging unit. Fluctuating discharges and/or injury to the cell were thus readily visible and these were immediately discarded. All stable visual thalamic and cortical responses were re-analysed off-line after histological confirmation of the recording and/or stimulation sites. Rate and interval histograms, frequency distributions, and spike counts were used to obtain

further characteristics of the discharging units. All such analyses were done using SPIKE2 customised software. All the peristimulus histograms displayed in this dissertation were obtained by converting and exporting the SPIKE2 histograms to a multi-purpose graphics package (Freelance Graphics by Lotus).

4.7. Histological Procedures

On completion of the electrophysiological recordings, the rat was overdosed with equithesin (~0.5 ml i.p.) and subsequently thoracotomised. The beating heart was exposed and a blunted perfusion needle (15G) was inserted into the left ventricle. The wall of the right atrium was cut so as to permit venous drainage. Immediately thereafter, approximately 300 ml of physiological saline (SABAX: NaCl 0.9gm%) was infused until the animal was almost completely exanguinated. The perfusate was then switched to a 10% phosphate buffered formalin solution (pH 7.4). About 500 ml of formalin was required for adequate fixation. Thereafter, the animal was decapitated and the head was immersed and stored in 80 ml of 10% phosphate buffered formalin for a minimum of 24 hrs.

After post-fixation, the dorsal and caudal parts of the cranium was carefully removed to expose the cortical surface. The bony sockets of the external auditory meatus were left intact. The lower jaw was also removed to facilitate optimal blocking of the tissue. Thereafter, the head was remounted in the stereotaxic frame in the same orientation as that used for the electrophysiological recordings. Using a curved scalpel blade attached to a manual microdrive, the brain was then blocked in the coronal plane at least 4 mm anterior (towards Bregma) to the LGN penetration and 4 mm posterior to the striate cortical penetration.

80-100μm coronal slices were then cut using the Vibratome Series 1000 Sectioning System. The brain slices were serially placed in multi-division trays containing 0.1 M phosphate buffer. Using a pair of fine paintbrushes, selected brain slices were carefully placed onto gelatinised slides and allowed to dry overnight. The sections were then stained with Cresyl Fast Violet. DPX mountant was used to secure the coverslips in place.

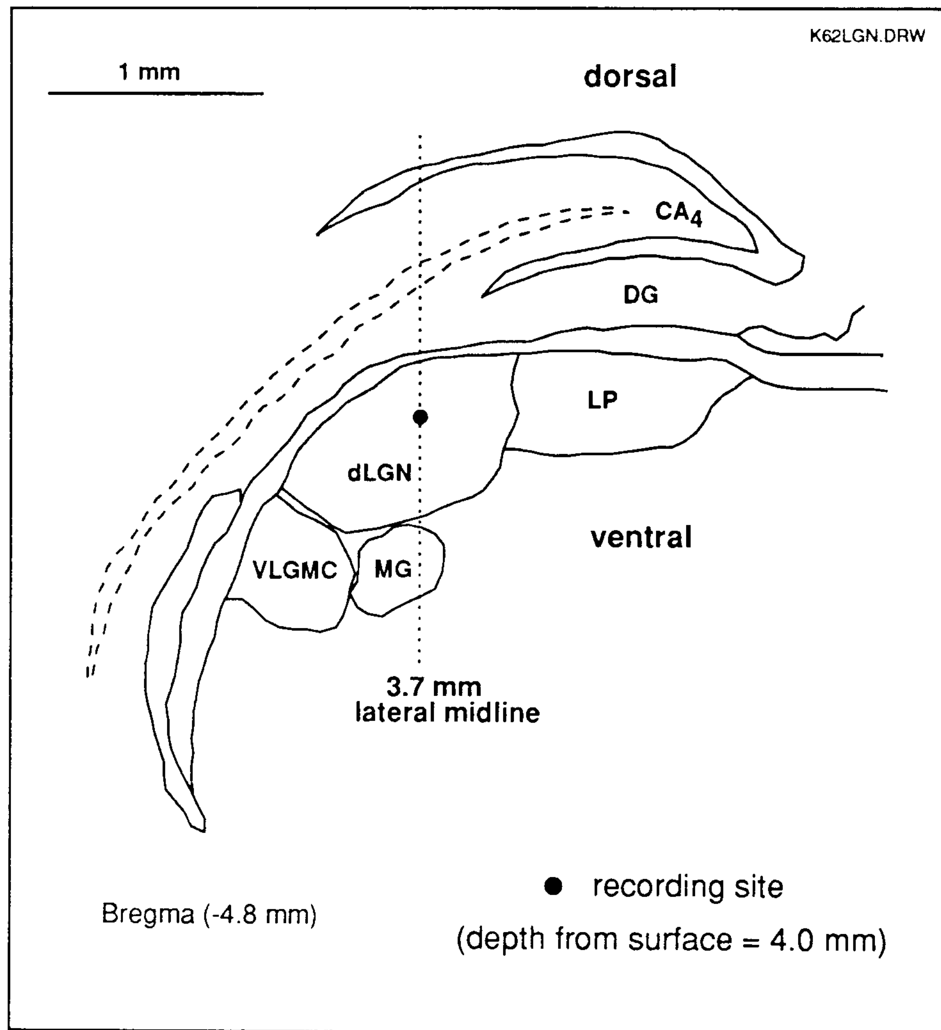


Fig. 4.3. A digitised camera lucida representation of the site of recording (filled circle) in the dLGN together with locations of some of the surrounding nuclei. The depth of the recording electrode was found to be 4 mm from the surface of cortex. The target coordinates were 4.8 mm posterior Bregma and 3.7 mm lateral to midline. Abbreviations: *dLGN* - dorsolateral geniculate nucleus; *LP* - lateral posterior thalamic nucleus (pulvinar); *MG* - medial geniculate nucleus; *VLGMC* - ventral lateral geniculate nucleus, magnocellular; CA_4 - field CA_4 of Ammon's horn, hippocampus; *DG* - dentate gyrus. Scale: as shown in figure.

The location of the recording and/or stimulating electrode was then confirmed by microscopic histological examination. The location of recording and stimulating sites were estimated on the basis of: (a) the electrode tract (especially for the thick stimulating electrode tracts in the LGN), (b) the vertical end-point of the tract corresponding to the tip of the electrode and, (c) the known depth of the electrode tip as measured from the microdrive. For the dLGN penetrations, landmarks like the ventral lateral geniculate nucleus

(VLMGC), medial geniculate nucleus (MG), lateral posterior thalamic nucleus (LP), dentate gyrus (DG) (see Fig. 4.3); and fields CA1, CA2, CA3, and CA4 of Ammon's horn were used to confirm the recording sites with reference to the stereotaxic co-ordinates of the atlas of Paxinos & Watson (1986). For the striate cortical penetrations and resulting electrode tracts, only the striated appearance of cortex, the changes in the density and thickness of the white matter, and the overall structure of the cortical sections between -7.3 to -8.3 mm (posterior Bregma) were used. The boundaries of the visual areas as depicted in the atlas of Paxinos & Watson (1986) are presumably based on the electrophysiological studies of other researchers that carefully mapped the visual areas. The sometimes poor yield of visual cortical responses obtained using Paxinos & Watson's target coordinates prompted us to also use coordinates that were based on the visuotopic maps of Espinoza & Thomas (1983).

Using the camera lucida technique with the Olympus BH-2 light microscope, some of the sections containing the cortical and/or geniculate electrode tracts were traced and later digitised. All clearly visible nuclei of the section that were in close proximity to the LGN were included (see Fig. 4.3). This provided further evidence that our recording electrodes were in the LGN, and not in any of the other thalamic nuclei.

Chapter 5

RESULTS & DISCUSSION - PART 1

CORTICAL RESPONSE TO TRANSIENT STIMULI

5.1. Introduction

This chapter focuses on the response of visual cortical neurones to transient electrical and strobe-flash stimulation. As highlighted earlier (see chapter 1), this series of experiments were motivated by a recent study of Douglas *et al* (1989). They found that upon pulsed electrical stimulation of the geniculo-cortical afferents (in the cat), the intracellular response of visual cortical cells consisted of a brief excitatory phase followed by a 200-300 ms inhibitory phase. This protracted period of inhibition was unexpected and might be dependent on the kind of stimulus applied. Electrical stimulation is unnatural because it provides a synchronous volley of excitation to the cortical network. The functional significance of this protracted inhibition is also difficult to explain. Furthermore, it contradicts current models of cortical processing that rely on strict serial processing, as initially hypothesized by Hubel & Wiesel (1962) and later Barlow (1972).

Like Douglas *et al* (1989), our approach here was to also use brief (< 1 ms) electrical stimuli applied to the thalamic afferents to record the temporal response of individual visual cortical neurones. In addition, we have recorded the response of these very same neurones to *natural* stimuli of similar duration i.e. < 1 ms stationary strobe flashes. One of the primary aims of this study was to compare the response of cortical neurones to transient electrical and strobe-flash stimulation. Electrical pulse stimulation is a popular engineering test signal. Biologically, even though electrical stimulation is unnatural, it might be valuable in analysing the synaptic circuitry of the cortex as it is highly reproducible and easily controlled. The advantage of pulse stimuli (flash or electrical) over conventional long-durationed stimuli e.g. spots, bars, checker-like blocks, and other pattern stimuli, is that it allows one to assess the performance of the circuit over time independent of any interference from the stimulus. So our approach was to trigger the cortical circuits and observe the evolution of the response of individual cortical neurones embedded in these circuits. A brief cortical response of a few milliseconds in duration i.e. behaving in a highly linear manner, will imply a

relatively simple transformation across the geniculo-cortical and/or intracortical circuits. On the other hand, a protracted cortical response (evolving over a few hundred milliseconds), be it excitatory, inhibitory or a combination of both, will be characteristic of a non-linear system reflecting a complex web of synaptic interactions. Indeed, the richly interconnected and complex microanatomy of the cortex (see chapter 3) supports the possible existence of non-linear dynamics in the neocortex. Previous studies were unable to detect or even envisage the presence of non-linear network processes because even though they sometimes obtained protracted cortical responses, the duration of the presented stimuli were just as protracted i.e. *at least 1 second* in duration. Further, in such instances, any coupling between excitatory and inhibitory phases of tens of milliseconds were completely masked as the logging time extended to a few seconds (thousands of milliseconds).

5.2. Methods for Optimal Positioning of the Cortical Recording Electrodes

We used the stereotaxic coordinates of the atlas of Paxinos & Watson (1986) and the visuotopic maps of Espinoza & Thomas (1983) to select our target coordinates in the primary visual cortex. Like the study of Espinoza & Thomas (1983), our pilot studies showed that the centre of the upper temporal visual fields corresponded to 8 mm posterior Bregma & 4 mm lateral to midline. Consequently, much of the data collected in this series of experiments were obtained in experiments where the starting penetration coordinates were 8 mm posterior Bregma & 4 mm lateral midline. Subsequent penetrations were made at 0.3 mm intervals anterior, posterior, medial, and lateral to the starting penetration. In addition, we found that cortical tissue wherein the neurones that had upper temporal visual fields, were bounded by two prominent arteries approximately 1.2 mm anterior and 0.8 mm posterior to 8 & 4 mm (posterior Bregma & lateral midline respectively). These arteries were often used as reference points i.e. during the burring procedure and removal of the dura mater, if these vessels were absent from view, then the burr-holes would be enlarged until these reference points were visible. Consequently, a compromise between using the starting penetration of 8 mm posterior Bregma and 4 mm lateral midline together with the anterior-posterior "centre" of these arteries proved to be very effective in obtaining visual responses characteristic of striate cortex.

3 to 5 penetrations were made in the visual cortex during each experiment. Each penetration commenced at the surface, and continued down to approximately 1.5 mm, or until fibre activity was encountered. No visual responses were obtained if the tip of the recording electrode was less than 0.2 mm from the surface. This might be expected as layer 1 is relatively cell free (Douglas & Martin, 1990).

5.3. Methods for Optimal Positioning of the Lateral Geniculate Nucleus (LGN) Stimulating Electrodes

The LGN tungsten stimulating electrodes were placed between 3.4 and 3.6 mm lateral to midline, and between 3.8 and 4.3 mm posterior Bregma. In addition to the above stereotaxic co-ordinates, the pattern of neuronal activity as the LGN is approached from the surface of the brain with the tungsten electrode was used to ensure the correct placement of the stimulating electrodes i.e. it was possible to record multi-unit extracellular activity through the tungsten electrode by attaching it to the headstage preamplifier (see chapter 4, section 4.3). Signals relayed via the audio amplifier showed the following characteristic changes: Cortical tissue was largely silent, hippocampus revealed characteristic large amplitude "cracklings" at approximately 2 mm from the surface, corresponding to CA1 of Ammon's horn (see atlas of Paxinos & Watson, 1986). Towards the ventral surface of hippocampus, faint multi-unit LGN responses were heard in the background. At 3.8 mm from the surface of cortex, characteristic "thrashing" multi-unit responses of the LGN were heard in response to visual stimuli i.e. spot stimuli repeatedly moved back and forth across the central visual fields. The responses usually started to deteriorate at 4.5 mm from the surface of the brain and disappeared completely at 4.8 to 5.0 mm. Consequently, the optimal depth for the placement of the tungsten stimulating electrodes was about 4 mm from the cortical surface. Once the tungsten stimulating electrodes were optimally positioned in the LGN, they were disconnected from the headstage preamplifier and connected to the electrical stimulus isolator unit (see chapter 4, section 4.5), ready for electrical pulse stimulation of the LGN.

5.4. General Characteristics and Observations

A total of 81 cortical units from 21 rats were recorded in this study. Of these, 61 (75%) neurones were visually responsive to strobe and/or hand-held stimuli (see Table 5.1). The remaining 25% of cortical neurones were classified as "non-visual" due to their unresponsiveness to strobe-flash, hand-held spot or bar stimuli. The response of some of these neurones to electrical stimulation of the geniculo-cortical afferents was also assessed (see section 5.5). To facilitate the visibility of any segregation of excitatory and inhibitory phases in the post-stimulus recordings, we did not record the response of non-spontaneous discharging cortical units. However, in conditions of stable anaesthesia, we found that the vast majority of cortical units encountered were discharging spontaneously i.e. firing in the absence of any deliberate sensory stimulation. No effort was made to quantify the class of quiescent units.

Table 5.1: Population of recorded visual and non-visual cortical neurones.

	Anaesthetic			Total
	Saffan i.v.	Equith i.p.	Equith i.v.	
Visual	11	5	45	61
Non-Visual	4	2	14	20
Total	15	7	58	81

(i.v. intravenous, i.p. intraperitoneal)
see Table 5.3 for complete data set.

No attempt was made to map-out detailed and precise receptive field properties of visual cortical cells i.e. whether they were simple, complex or hypercomplex; and/or the degree of direction and orientation selectivity, as that demonstrated by the studies of Hubel & Wiesel (1962). This kind of approach has been exhausted and is now of little value in attempting to understand the cortical microcircuits (see chapter 3). However, the *location* of the overall receptive field of individual visual units was always carefully assessed with hand-held spot or bar stimuli, and the strobe unit (used for delivering the flash stimuli) was always positioned optimally, within the receptive field.

5.5. Response to Electrical Stimulation

The response to electrical pulse stimulation of the dorso-lateral geniculate nucleus (dLGN), and therefore presumably the geniculocortical afferents, were tested in 20 of the 64 (57 + 7) cortical units summarised in Table 5.1 above. All these recordings were obtained from equithesin anaesthetised rats (see Table 5.2 for complete data set). 19 of the 20 tested units were found to be responsive to strobe-flash (natural) stimulation also. These will be considered later in this chapter. In 17 of the tested 20 cells, bipolar electrical stimulation was used. In the remaining three, monopolar electrical stimulation was used. Electrical impulses were always 0.2 ms in duration and their amplitudes ranged from 80-400 μA (see Table 5.2).

Six typical cortical responses to pulsed electrical stimulation are shown in Fig. 5.1 (A-F). In this and subsequent examples, the data is presented in the form of peri-stimulus time histograms (PSTHs). As outlined in the methods (chapter 4), these represented the cumulative response over 40-80 trials. Each trial consisted of a control and test period. In all of the PSTHs presented, the vertical axis represents the number of spikes (action potentials). Since all of the tested units were discharging spontaneously (as evident in the control periods), inhibition (in the test period) has been operationally defined as the absence of spikes. The response of cells A, B, & C were recorded upon monopolar stimulation of the dLGN while the response of cells D, E, & F were obtained using bipolar stimulation of the dLGN. Electrical stimulation produced a characteristic pattern in all cortical cells, even in those cortical units that were not responsive to natural (visual) stimuli. The pattern of this response was always that of protracted inhibition, 197 ± 61 ms (mean ± sem) in duration [range 100 - 320 ms - see Table 5.2], irrespective of whether the stimulation was mono- or bi- polar (see Fig. 5.1). The protracted period of inhibition was often followed by a phase of rebound excitation (100-200 ms in duration), which completed the response.

Table 5.2: Cortical response to electrical stimulation of the LGN.

Key:

DEPTH: Depth of recording electrode in cortex.

STIM TYPE: Either monopolar or bipolar stimulation of the LGN used.

RANGE: Range of stimulus currents used to test the response to electrical stimulation.

THRESH: Threshold current.

OPTIM: Optimal current.

ES: Whether there were any early orthodromic or antidromic spikes. n = no, y = yes. Where recorded, latencies given in milliseconds.

DUR INHIB: Duration of sustained inhibition in the test period.

REBOUND: Whether there was a rebound phase of excitation that followed the sustained inhibition.

Notes: All 20 units tested under equithesin anaesthesia. Early orthodromic or antidromic spikes were visible only with bipolar electrical stimulation. Cells logged under several stimulus strengths, but only data of optimal stimulus strengths shown here.

	A	B	C	D	E	F	G	H	I	J
1	*Cx*	*resp*	*to elec*	*stim*						
2	**REF**	**CN**	**DEPTH**	**STIM TYPE**	**RANGE**	**THRESH**	**OPTIM**	**ES**	**DUR INHIB**	**REBOUND**
3	el2k38	1	0.8	monopolar		130		n	190	y
4	ec3k38	2	0.7	monopolar		110	130	n	190	y
5	el5k41	3	0.88	monopolar			132	n	180	y
6	cx41h01	4	0.75	bipolar	100-400	100	200	y (anti 2 ms)	150	y
7	cx42h05	5	0.65	bipolar			400	y (anti 2ms)	100	n
8	cx45h01	6		bipolar			350	y (ortho)	220	y
9	cx47h04	7	1.3	bipolar	300-400	300	400	n	100	y
10	cx48h05	8	0.61	bipolar	100-250	200	250	y (ortho 20ms)	320	y
11	cx52h03	9	0.77	bipolar	50-500	300	400	y (ortho 10ms)	250	y
12	cx53h11	10	1.02	bipolar	50-100	50	100	y (ortho 9ms)	160	y
13	cx55h07	11	1.5	bipolar	50-400	300	400	y (ortho 11ms)	180	y
14	cx56h02	12	0.78	bipolar	30-300	180	200	y (anti 2.5ms)	300	n
15	cx57h02	13	0.95	bipolar	100-500	200	300	n	300	y
16	cx65h07	14	0.7	bipolar	30-300	200	300	y (ortho 7.5ms)	220	y
17	cx66h06	15	0.77	bipolar	50-250	200	250	n	240	y
18	cx71h08	16	0.85	bipolar	50-100	100	100	n	160	y
19	cx73h06	17	1.1	bipolar	50-300	200	200	y (ortho 6ms)	180	y
20	cx74h08	18	0.75	bipolar	50-200	80	100	n	220	y
21	cx77	19	1.3	bipolar	100-400	300	400	y (5ms)	120	y
22	cx80	20	1	bipolar	200-400	300	400	y (3 & 10 ms)	150	y

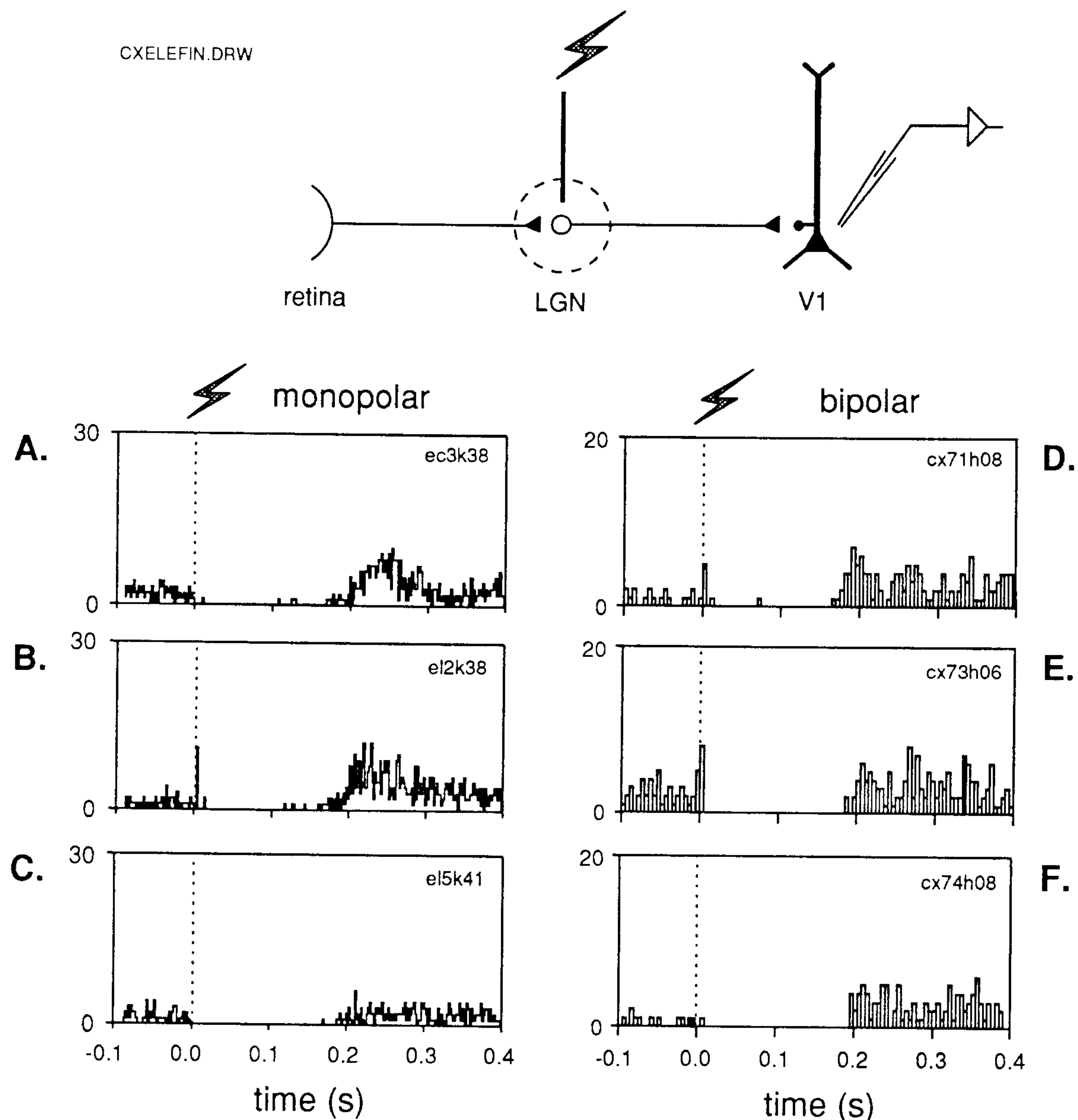


Fig. 5.1. Typical extracellular responses of 6 striate cortical cells to electrical stimulation of the ipsilateral dLGN (**A**-**F**). Data in this and subsequent examples presented in the form of peri-stimulus time histograms (PSTHs). Schematic (shown above the 6 PSTH examples) represents the experimental protocol of the stimulating and recording electrode arrangement. Mono- or bi- polar stimulating electrode positioned in the LGN, recording electrode positioned in the visual cortex (V1). Response of cells **A**-**C** were obtained upon monopolar stimulation of the dLGN (132 μA). Response of cells **D**-**F** were obtained with bipolar stimulation of the dLGN (100, 200, 100 μA for cells D, E, & F respectively). Electrical stimulation (applied at time zero) always produced a protracted period of inhibition (197 ± 61 ms) in all of the tested units, irrespective as to whether the stimulation was monopolar or bipolar. A 100-200 ms period of rebound excitation completed the cortical response. 100 ms control period precedes the test period. Application of electrical stimuli represented by dashed line (at time zero).

Monopolar stimulation evoked a large stimulus artefact lasting 5-10 ms, and so no *neural* events could be logged during that time window. Since the anticipated monosynaptic latency from the LGN to the visual cortex is 6-10 ms (Douglas & Martin, 1990b, and our observations here), we cannot reliably comment on early synaptic events in these recordings (A, B, & C in Fig. 5.1). Note that the monopolar stimulus artefact does not always contribute to the PSTH (A & C in Fig. 5.1). This is because the presence of the artefact in the PSTH depends on whether the artefact fell within the discriminator window or not. Thus, a very large, or small artefact might fall outside those limits, and so not be reflected in the PSTH. In such instances, the absence of early excitatory events during the time that the artifact is expected to be present (i.e. within 5-10 ms of stimulation) does not necessarily imply inhibition.

The primary advantage of bipolar stimulation is that it produces a smaller and shorter stimulus artefact. This ensures the visibility of any early orthodromic or antidromic spikes in the extracellular recordings. We found that with bipolar stimulation, such early ortho- or anti- dromic spikes often (12/17 cells - 71%) preceded the protracted inhibition. These spikes were best visible in the expanded time histograms i.e. analysing just the first 20 ms of the post-stimulus response (e.g. see Fig. 5.2 B).

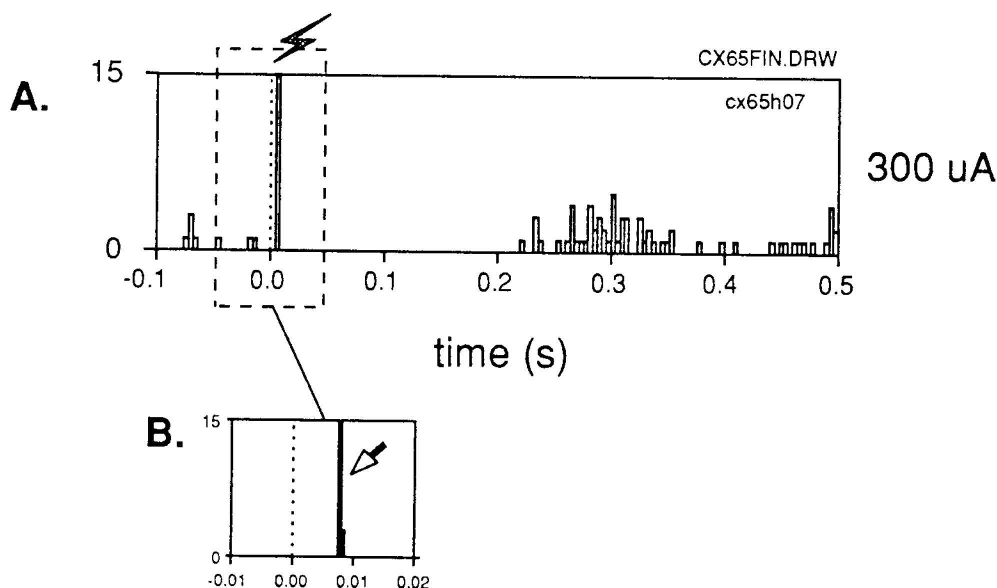


Fig. 5.2. Extracellular response of a single cortical neurone to bipolar electrical stimulation of the LGN (**A**). Expanded time view (**B**) shows an early orthodromic spike (arrowed), 7.5 ms latency, precedes the sustained (220 ms) inhibition. Stimulus strength = 300 μA. The only advantage of using bipolar stimulation in preference to monopolar stimulation is the smaller and shorter stimulus artefact. It is for this very reason that such early orthodromic (or antidromic) spikes are observed with bipolar stimulation.

Fig. 5.3, a coronal section, shows the tract from a monopolar stimulating electrode penetration, the end of which corresponds to the tip of the electrode and lies on the dorsal surface of the dLGN. The extracellular responses of cells A, B & C (Fig. 5.1) were obtained as a result of passing a current of 130 μA through a single tungsten electrode (i.e. monopolar), the tract of which is depicted in Fig. 5.3, confirming the correct placement of the stimulating electrode. Based on the estimates of Ranck (1981), the radius of stimulation (in the dLGN) extends to 500μm away from the tip of the cathodal stimulating electrode (arrowed in Fig. 5.3). The exact shape of the current field is believed to be very complex and will not be considered further (Ranck, 1981; Douglas, 1991 personal communication). Assuming the shape of the dorso-lateral geniculate nucleus to be cylindrical, we estimate the total volume of dLGN tissue to be 1.26 mm^3 ($V = \pi R^2 h$). Assuming that the spread of electrical stimulation allows for the volume of stimulation to be spherical, 0.4 mm^3 of tissue was stimulated ($V = \pi R^3$). This represents 32% (0.4/1.26) of the total dLGN volume.

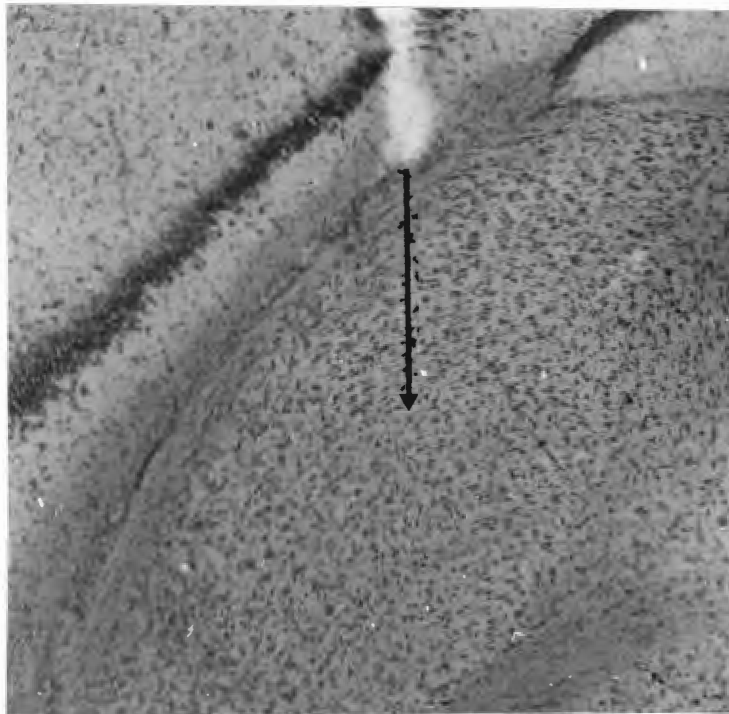


Fig. 5.3. Coronal section showing the dorsal lateral geniculate nucleus (dLGN). The lesion of the monopolar stimulating electrode is clearly visible, the tip of which rests on the dorsal surface of the dLGN. PSTHs **A**, **B** and **C** shown in Fig. 5.1 were obtained as a result of passing a current of 132 μA through a stimulating electrode, the lesion of which corresponds to that shown in this section. The radius of tissue that was stimulated with this current strength was estimated to be 500 μm, as represented by the solid vertical arrowed line. This section was cut at a thickness of 80μm and stained with cresyl fast violet.

5.6. Response to Strobe-Flash Stimulation

The response to flash stimulation was recorded in 61 visually responsive cortical neurones (see Table 5.3 for complete data set). The response of 20 of these cells to electrical pulse stimulation was also recorded (see section 5.5 above). The responses to flash stimulation (as presented in this section) were obtained from rats that were anaesthetised with either equithesin or saffan (see chapter 4 for details of methods).

Six typical cortical responses to flash pulse stimulation are shown in Fig. 5.4 (A-F). While these are representative of the entire sample of recorded visually responsive units to flash stimulation from equithesin anaesthetised animals, further examples are given in Fig. 5.5, 5.8, 5.9, 5.10, 5.11, 5.12, 5.13 & 5.14. These will be discussed later in this chapter.

The animals used to record the extracellular responses of cells A, B, & C in Fig. 5.4 were anaesthetised with equithesin administered intraperitoneally (i.p.). The extracellular responses of cells D, E, & F in Fig. 5.4 were recorded from animals anaesthetised with equithesin administered intravenously (i.v.). Flash stimulation induced protracted excitatory events (extending to a few hundred milliseconds) in all of the cortical neurones recorded (see Table 5.3). These were often coupled with multiple bouts of short-durationed inhibition. Protracted excitatory events were visible with both routes of equithesin administration. More specifically, application of the strobe flash to the contralateral eye at time zero, usually initiated a prominent excitatory discharge after a latency of 30-60 ms (Fig. 5.4) [see Table 5.3 and its associated scatter plot]. Thereafter, for the next 20-40 ms, either inhibition (cells B, E, & F) or return to basal firing levels (cells C & D) was observed. Secondary excitatory peaks completed the response (arrowed in Fig. 5.4). From here on, we refer to the initial excitatory peaks as primary excitation because they are almost always larger in magnitude than the secondary excitatory ones. The data show that no sustained flash-evoked *inhibition* (hundreds of milliseconds in duration), precedes or follows the primary excitatory phase.

Two additional cortical responses from equithesin anaesthetised rats are shown in Fig. 5.5. These two cells depicted trends of excitation (and inhibition) similar to that described in the previous examples. That the duration of the secondary inhibition might be modulated under certain conditions will be discussed later in the chapter.

Table 5.3: Cortical response to flash stimulation.

Key:

REF: Reference number used to log the cell's response under a specific test condition.

CN: Cell number.

RN: Rat or experiment number.

ANAES: Type of anaesthesia. i.p. equi - intraperitoneal injection of equithesin, i.v. equi - intravenous administration of equithesin.

ILLUM: Level of surround or background illumination (measured in lux).

DEPTH: Depth of the recording electrode in the cortex.

SACP: Spontaneous activity in the control period.

LAR: Latency to primary response.

DUR: Duration of overall response to the flash pulse.

NV: non-visual units.

NA: not applicable.

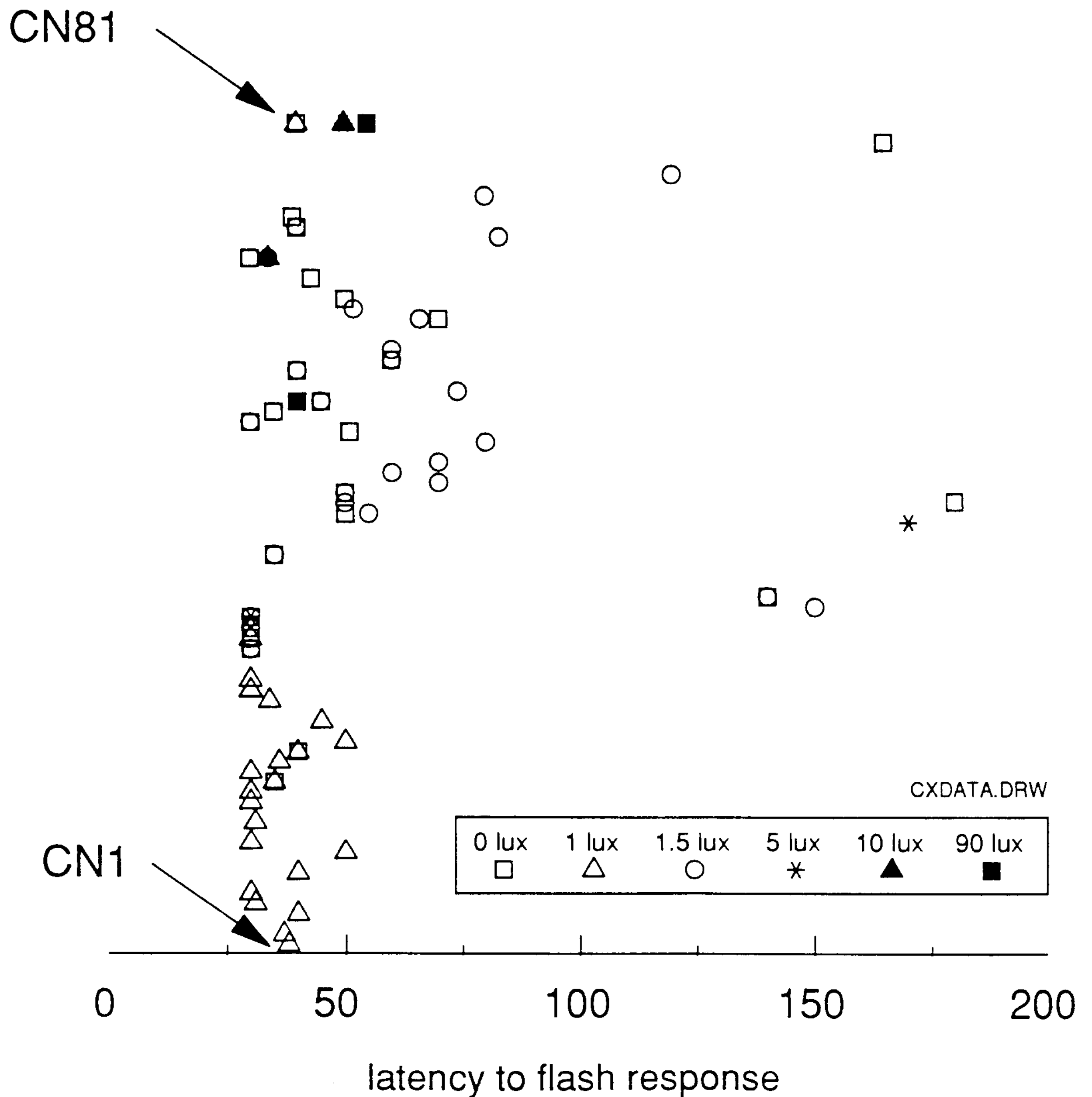
Notes: 17/21 (85%) of tested cortical neurones were found to be sensitive to changes in the level of background illumination.

cortex	*single*	*flash*	*data*					
REF	**CN**	**RN**	**ANAES**	**ILLUM**	**DEPTH**	**SACP**	**LAR**	**DUR**
T1C2K27	1	27	i.p. equi	1	0.88	y	38	362
C3STK37	2	37	i.p. equi	1	0.7	Y	37	273
NV	3	37	i.p. equi	1	1	Y	NA	NA
SR2K38	4	38	i.p. equi	1	0.8	Y	40	330
SB3K38	5	38	i.p. equi	1	0.7	Y	31	369
FL5K41	6	41	i.p. equi	1	0.88	Y	30	370
NV	7	41	i.p. equi	1	0.81	Y	NA	NA
CX05H06	8	85	i.v. equi	1	0.5	Y	40	>500
CX06H01	9	86	i.v. equi	1	0.62	Y	not	logged
CX06H03	10	86	i.v. equi	1	0.56	Y	50	370
CX08H00	11	89	i.v. saffan	1	0.5	Y	30	390
NV	12	89	i.v. saffan	1	1.25	Y	NA	NA
CX08H04	13	89	i.v. saffan	1	1.5	Y	31	279
NV	14	89	i.v. saffan	1	1.6	Y	NA	NA
CX08H05	15	89	i.v. saffan	1	0.55	Y	30	>500
CX08H10	16	89	i.v. saffan 0.25 ml	1	1	Y	NA	NA
CX08H11	16	89	i.v. saffan	1	1	Y	30	>500
CX08H12	16	89	i.v. saffan 0.25 ml	1	1	N	NA	NA
CX09H02	17	90	i.v. saffan	1	0.75	Y	35	240
CX09H05	17	90	i.v. saffan	90	0.75	Y	no resp	NA
CX09H06	17	90	i.v. saffan	1	0.75	Y	35	365
CX09H07	17	90	i.v. saffan	0	0.75	Y	35	>500
CX09H08	18	90	i.v. saffan	1	0.93	Y	30	>500
CX11H03	19	94	i.v. saffan	1	1.02	Y	36	>500
CX12H00	20	94	i.v. saffan	1	1.05	Y	40	>500
CX12H01	20	94	i.v. saffan	0	1.05	Y	40	>500
CX12H02	20	94	i.v. saffan	1	1.05	Y	40	>500
CX13H02	21	96	i.v. saffan	1	0.48	Y	50	>500
NV	22	96	i.v. saffan	1	1.16	Y	NA	NA
CX15H05	23	96	i.v. saffan	1	1.17	Y	45	>500
NV	24	96	i.v. saffan	1	1.21	Y	NA	NA
CX14H01	25	97	i.v. saffan	1	0.3	Y	34	>500
CX25H08	26	102	i.v. equi	1	0.3	Y	30	>500
CX26H03	27	102	i.v. equi	1	0.35	Y	30	>500
CX27H00	28	102	i.v. equi	1	0.2	N	NA	NA
CX28H00	29	102	i.v. equi	1	0.85	N	NA	NA
CX29H00	30	102	i.v. equi	1.5	0.98	Y	30	>500
CX29H01	30	102	i.v. equi	1.5	0.98	Y	30	>500
CX29H02	30	102	i.v. equi	0	0.98	Y	30	>500
CX29H03	30	102	i.v. equi	1.5	0.98	Y	30	>500
CX29H05	30	102	i.v. equi	1.5	0.98	Y	30	>500

CX30H00	31	102	i.v. equi	1	1.02	Y	30	>500
CX30H02	31	102	i.v. equi	1	1.02	Y	30	>500
CX30H03	31	102	i.v. equi	1	1.02	Y	30	>500
CX30H04	31	102	i.v. equi	0	1.02	Y	30	>500
CX31H00	32	102	i.v. equi	1.5	1.08	Y	30	>500
CX31H01	32	102	i.v. equi	1.5	1.08	Y	30	>500
CX31H02	32	102	i.v. equi	0	1.08	Y	30	>500
CX34H01	33	106	i.v. equi	0	0.75	Y	30	>500
CX34H02	33	106	i.v. equi	1.5	0.75	Y	30	>500
CX34H03	33	106	i.v. equi	5	0.75	Y	30	>500
CX34H04	34	106	i.v. equi	1.5	1	N	150	>500
CX36H00	35	106	i.v. equi	1.5	1.2	Y	140	>500
CX36H01	35	106	i.v. equi	0	1.2	Y	140	>500
CX37H00	36	106	i.v. equi	1.5	0.8	Y	lost in	logging
CX38H00	37	106	i.v. equi	1.5	1	Y	lost in	logging
CX39H00	38	106	i.v. equi	1.5	1.2	Y	lost in	logging
CX41H04	39	111	i.v. equi	1.5	0.75	Y	35	>500
CX41H05	39	111	i.v. equi	0	0.75	Y	35	>500
CX41H07	39	111	i.v. equi	5	0.75	Y	no resp	at this illum
CX41H08	39	111	i.v. equi	90	0.75	Y	no resp	at this illum
CX41H09	39	111	i.v. equi	1.5	0.75	Y	35	>500
CX41H10	39	111	i.v. equi	0	0.75	Y	30	>500
NV	40	111	i.v. equi	1.5	0.81	Y	NA	NA
NV	41	111	i.v. equi	1.5	0.87	Y	NA	NA
CX42H02	42	111	i.v. equi	5	0.65	N	170	>500
CX46H02	43	111	i.v. equi	1.5	1.18	Y	55	>500
CX46H03	43	111	i.v. equi	0	1.18	Y	50	>500
CX47H00	44	115	i.v. equi	1.5	1.3	N	50	>500
CX47H01	44	115	i.v. equi	0	1.3	N	180	>500
CX47H02	44	115	i.v. equi	1.5	1.3	N	50	>500
CX47H03	44	115	i.v. equi	0	1.3	N	190	>500
CX48H00	45	115	i.v. equi	1.5	0.61	N	50	>500
CX48H01	45	115	i.v. equi	0	0.61	N	50	>500
CX48H06	45	115	i.v. equi	0	0.61	N	50	>500
CX48H07	45	115	i.v. equi	1.5	0.61	N	50	>500
CX49H01	46	116	i.v. equi	1.5	0.45	Y	70	>500
CX49H06	47	116	i.v. equi	1.5	0.8	Y	60	>500
CX51H00	48	116	i.v. equi	1.5	0.85	Y	70	>500
NV	49	116	i.v. equi	1.5	0.9	Y	NA	NA
CX52H00	50	118	i.v. equi	1.5	0.77	Y	80	150
CX53H01	51	118	i.v. equi	0	1.02	Y	51	>500

CX54H00	52	118	i.v. equi	1.5	?	Y	30	400
CX54H01	52	118	i.v. equi	0	?	Y	30	295
CX55H01	53	118	i.v. equi	0	1.5	Y	35	>500
CX56H00	54	120	i.v. equi	1.5	0.78	Y	45	400
CX56H01	54	120	i.v. equi	0	0.78	Y	45	400
CX56H16	54	120	i.v. equi	90	0.78	Y	40	300
CX56H17	54	120	i.v. equi	5	0.78	Y	40	>500
CX56H18	54	120	i.v. equi	1.5	0.78	Y	40	>500
CX56H25	55	120	i.v. equi	1.5	0.95	Y	74	>500
CX56H26	55	120	i.v. equi	0	0.95	N	no resp	no resp
NV	56	120	i.v. equi	1.5	1.25	?	NA	NA
CX63H01	57	122	i.v. equi	1.5	0.44	N	40	>500
CX63H02	57	122	i.v. equi	0	0.44	N	40	>500
CX64H00	58	122	i.v. equi	1.5	0.55	Y	60	>500
CX64H01	58	122	i.v. equi	0	0.55	Y	60	>500
CX64H02	58	122	i.v. equi	1.5	0.55	Y	60	>500
CX65H00	59	122	i.v. equi	1.5	0.24	N	60	>500
NV	60	122	i.v. equi	1.5	0.33	?	NA	NA
NV	61	122	i.v. equi	1.5	1.06	?	NA	NA
CX66H01	62	123	i.v. equi	0	0.77	Y	70	285
CX66H08	62	123	i.v. equi	1.5	0.77	Y	66	250
CX67H02	63	123	i.v. equi	1.5	0.82	Y	52	373
CX68H00	64	123	i.v. equi	0	0.83	Y	50	350
NV	65	123	i.v. equi	1.5	0.9	Y	NA	NA
CX69H03	66	123	i.v. equi	0	1.18	Y	43	>500
NV	67	123	i.v. equi	1.5	1.35	?	NA	NA
CX71H01	68	123	i.v. equi	0	0.85	Y	30	>500
CX71H02	68	123	i.v. equi	1.5	0.85	Y	34	>500
CX71H05	68	123	i.v. equi	10	0.85	Y	34	>500
CX71H03	68	123	i.v. equi	0	0.85	Y	30	>500
NV	69	123	i.v. equi	1.5	0.92	?	NA	NA
CX72H01	70	123	i.v. equi	1.5	0.95	Y	83	30
CX73H00	71	123	i.v. equi	1.5	1.18	Y	40	>500
CX73H01	71	123	i.v. equi	0	1.18	Y	40	>500
CX73H04	71	123	i.v. equi	1.5	1.18	Y	40	>500
CX74H01	72	123	i.v. equi	0	0.75	Y	39	>500
CX74H02	72	123	i.v. equi	1.5	0.75	Y	no spike	>500
CX74H03	72	123	i.v. equi	0	0.75	Y	39	>500
NV	73	123	i.v. equi	1.5	1.12	Y	NA	NA
CX75H01	74	123	i.v. equi	1.5	1.2	Y	80	>500

NV	75	124	i.v. equi	1.5	0.52	?	NA	NA
CX77H01	76	124	i.v. equi	1.5	1.3	N	120	>500
NV	77	124	i.v. equi	1.5	0.66	Y	NA	NA
NV	78	124	i.v. equi	1.5	0.83	?	NA	NA
CX79H01	79	124	i.v. equi	0	0.95	Y	165	147
NV	80	124	i.v. equi	1.5	0.65	?	NA	NA
CX80H01	81	124	i.v. equi	0	1.1	Y	40	>500
CX80H03	81	124	i.v. equi	5	1.1	Y	50	>500
CX80H04	81	124	i.v. equi	10	1.1	Y	50	>500
CX80H05	81	124	i.v. equi	90	1.1	Y	55	>500
CX80H06	81	124	i.v. equi	0	1.1	Y	40	>500



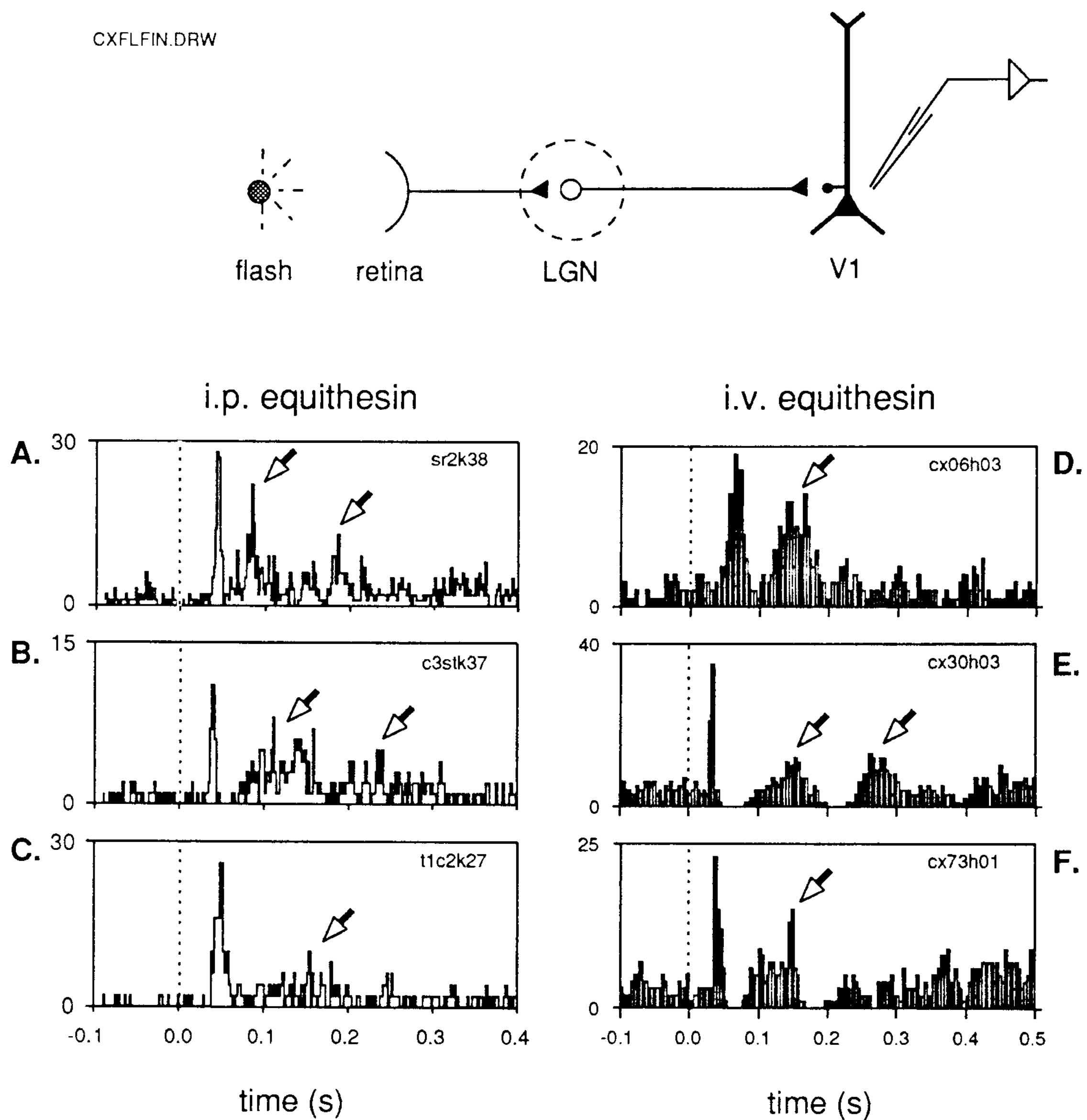


Fig. 5.4. Extracellular responses of 6 cortical neurones to strobe-flash stimulation of the contralateral eye (A-F). Response of cells **A-C** were recorded from animals anaesthetised with equithesin administered intraperitoneally. Response of cells **D-F** were recorded from animals anaesthetised with equithesin administered intravenously. Irrespective of the route of equithesin administration, flash stimulation produced a sustained excitatory response in all of the tested visually responsive units. The flash stimuli initiated a primary excitatory phase after a latency of 30-60 ms. Apart from this primary "burst" of excitation, secondary excitatory events (arrowed) were always present. These were often coupled with small bouts of inhibition (see E & F). Background illumination kept at < 1 lux for all recordings. Flash stimuli in all cases applied at time zero (as indicated by the dashed line).

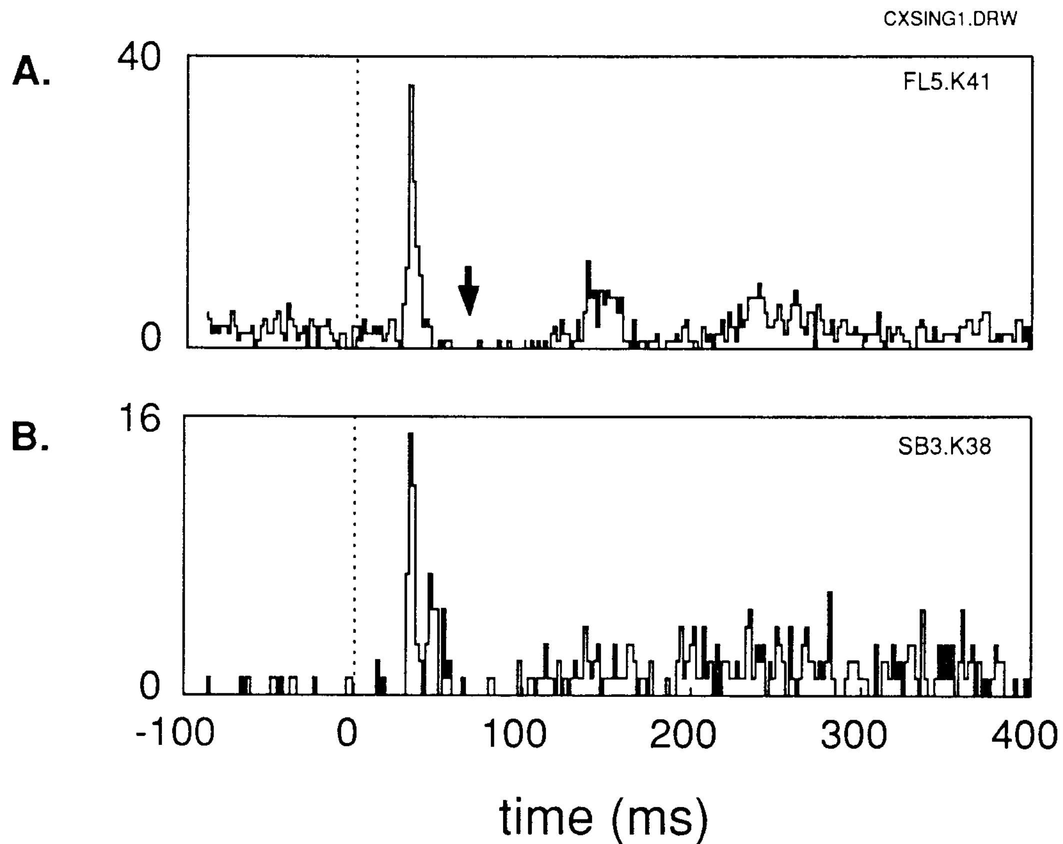


Fig. 5.5. Extracellular responses of 2 additional cortical neurones to strobe-flash stimulation recorded from equithesin anaesthetised rats. Like those examples shown in Fig. 5.4, flash stimuli evoked a protracted response of coupled excitation and inhibition. Cell A showed a distinct secondary inhibitory phase (arrowed) following the prominent early excitatory spike. In cell B, while there were no prominent secondary excitatory peaks, the level of activity that followed the early spike was markedly higher than that in the control period.

A similar evolution of cortical re-excitation (in response to flash stimulation) was also evident in saffan anaesthetised rats. We chose to record cortical responses with this anaesthetic, in addition to equithesin anaesthetised rats, for the following reasons:

- to dispel any doubt that the extended cortical responses were an artefact of barbiturate (i.e. equithesin) anaesthesia,
- saffan (alphaxalone-alphadolone), a steroid based drug, has been used successfully in electrophysiological studies in the cat (Douglas *et al*, 1991; Douglas & Martin, 1991), and we explored its value for electrophysiological studies in the rat.

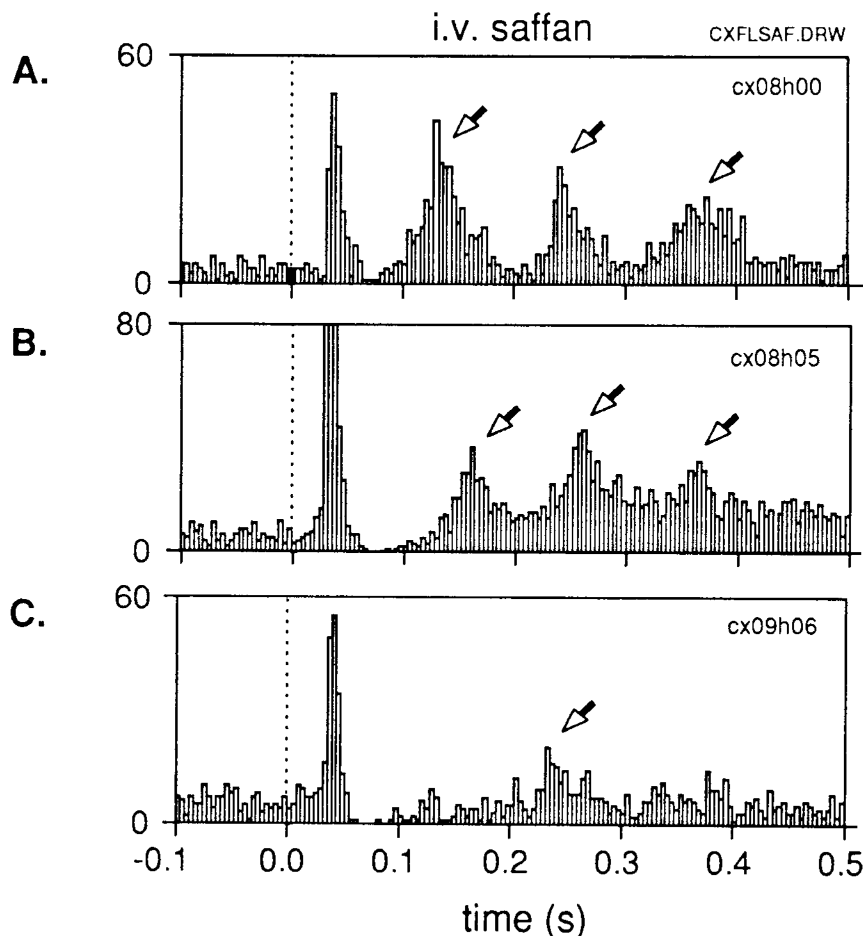


Fig. 5.6. Extracellular responses of 3 cortical neurones to strobe-flash stimulation of the contralateral eye. All 3 of these cells were obtained from saffan-anaesthetised rats. Like the equithesin-anaesthetised rats, flash stimulation produced primary and secondary excitatory bouts of excitation lasting 400-500 ms. Latency to primary excitation in these three examples were 25-35 ms. Open arrows indicate secondary bouts of re-excitation. Background illumination kept at around 1 lux for all recordings. As in previous example, flash stimuli indicated by dashed line at time zero.

Of the 60 visually responsive cortical neurones presented in this section, only 11 (18%) were obtained from saffan-anaesthetised rats (see Table 5.1 & 5.3). The remaining 50 cells were recorded from equithesin anaesthetised rats. When a visual unit was located in our saffan anaesthetised rats, the profile of the response, was qualitatively very much similar to that encountered in our equithesin anaesthetised animals.

Three typical cortical responses to flash pulse stimulation from saffan anaesthetised animals are shown in Fig. 5.6 (A-C). On application of the strobe-flash, a primary excitatory phase emerged after a latency of 25-50 ms. Thereafter, a 20-30 ms quiescence or return to basal activity was evident. As often seen in the equithesin anaesthetised rats, repeated bouts of excitation (extending to a few hundred milliseconds) completed the response (see Fig. 5.6).

An important feature of using saffan anaesthesia was the immense control of the level of the discharge of the tested visual units. We found that the responsiveness of the recorded cortical neurones could be repeatedly and reversibly modulated by infusing bolus injections (0.2-0.25 ml) of the drug intravenously. In such instances, a visual cortical unit's activity to flash stimulation could be switched-off within seconds of the drug infusion and then returned to its original discharge pattern within 5 minutes from the drug infusion (see Fig. 5.7). Such fine control was not possible with equithesin anaesthesia, even when equithesin was administered intravenously.

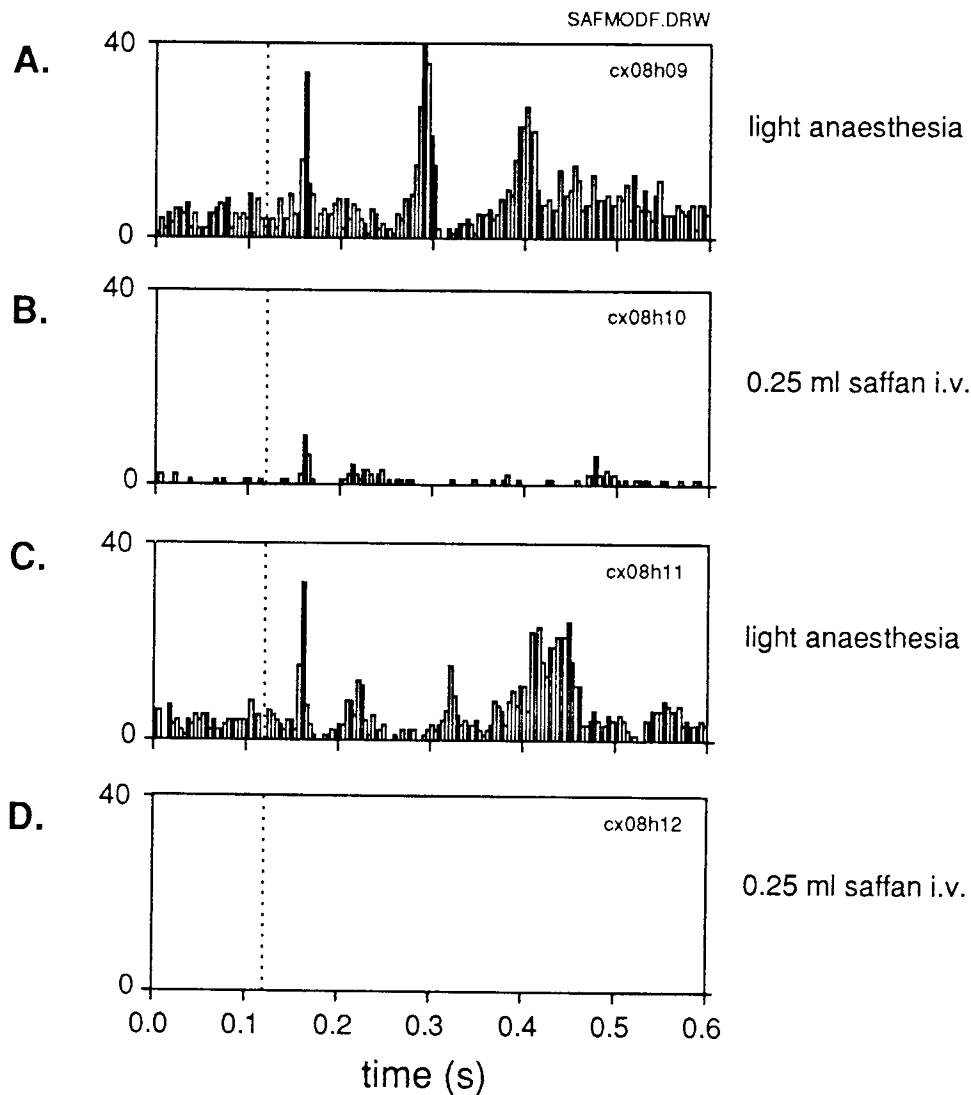


Fig. 5.7. Extracellular responses of a single cortical neurone to flash stimulation under different levels of saffan anaesthesia. This example demonstrates the rapid reversibility of this anaesthetic agent on the responsiveness of the cortical unit. Stimulus indicated by dashed line. **A.** Flash response in light anaesthesia. As observed in previous examples, cortical re-excitation was evident. **B.** Flash response of the same unit after a 0.25 ml bolus injection of saffan (i.v). Response recorded after 1 min from injection. The response of the unit to flash stimulation was markedly attenuated. **C.** Flash response of the same unit after a 10 minute recovery period from the 0.25 ml injection. While the profile of the response is not identical to A, the extended excitatory phases re-emerge. **D.** Flash response after another 0.25 ml bolus injection of saffan. This time, the unit was switched-off completely.

It is not possible to further classify or categorise the recorded population of visual cortical units in terms of their temporal profiles. This is because no two cortical neurones respond in the same manner i.e. the degree of excitation and/or coupled inhibition varies from one cell to another. That such variation might be due to fluctuating levels of anaesthesia was minimised by infusing the anaesthetic agents continuously via the intravenous route. Hubel (1988) also reported that different visual cortical neurones do not necessarily respond in the same manner even though they might have similar receptive field structures. The common feature in all of the visually responsive, spontaneously discharging cortical units recorded in this study, was the extended cortical re-excitatory response that continues for 200-400 ms, even though the stimuli were always less than a millisecond in duration.

Apart from the level of anaesthesia, we suspected that changes in the surround (or background) illumination might also influence the temporal profile of the cortical response to flash stimulation. This is because an earlier study (Creutzfeldt *et al*, 1969), albeit using cats, found that the temporal response to flash stimulation of some visual cortical units could be modulated by changing the level of background illumination (see chapter 3, section 3.3.2 for further details). All of the preceding data were recorded from animals where the surround illumination was kept around 1 lux. In the following series of experiments, we tested the response of 21 cortical neurones to strobe-flash stimulation, where the response of *each* neurone was recorded with different surround illuminations (0, 1.5, 10, and sometimes 90 lux). All 20 of these neurones were recorded only from rats receiving continuous intravenous administration of equithesin or saffan.

17 of the 21 tested cortical neurones (81%) were found to be sensitive to the changes in the level of the background illumination (see Table 5.3). In this section, we present six examples that are fully representative of this sample of 17. The remaining 19% (4 of 21) of visual units whose flash-evoked responses were insensitive to the level of background illumination receive no further consideration.

This sensitivity of the flash-evoked response to the level of background illumination was reflected primarily as an increase in the duration of a *secondary* inhibitory phase. Specifically, the duration of the secondary inhibitory phase was greatest at the lowest level of surround illumination (0 lux) (see Fig. 5.8, 5.9, 5.10, & 5.11). Of the 16 visual units that responded with a primary excitatory phase, 10 (62%) displayed secondary inhibitory

phases at post-zero (1.5-10 lux) levels of surround illumination. Typical examples are shown in Fig. 5.8 & 5.10. The remaining 6 neurones did not show any flash-evoked secondary inhibitory phases at post-zero levels of background illumination. Two examples are shown in Fig. 5.9 & 5.11. Nevertheless, in *all* 16 of these units, the duration of flash-evoked inhibition increased by 40-70 ms, from post-zero (10 & 1.5 lux) to zero (0 lux) levels of background illumination. So, if a particular cell displayed a secondary inhibitory phase of 60 ms at higher levels of surround illumination i.e. 1.5 & 10 lux, then at the lowest level of surround illumination (0 lux), that cell displayed an extended secondary inhibitory phase of 80-100 ms.

In only 1 of the tested 20 cells, did a *primary* inhibitory phase emerge at the lowest level of background illumination (0 lux) (Fig. 5.13 B & D). This inhibition was followed by two bouts of coupled excitation and inhibition. At post-zero levels of background illumination, no primary or secondary inhibition emerged (Fig. 5.13 A & C).

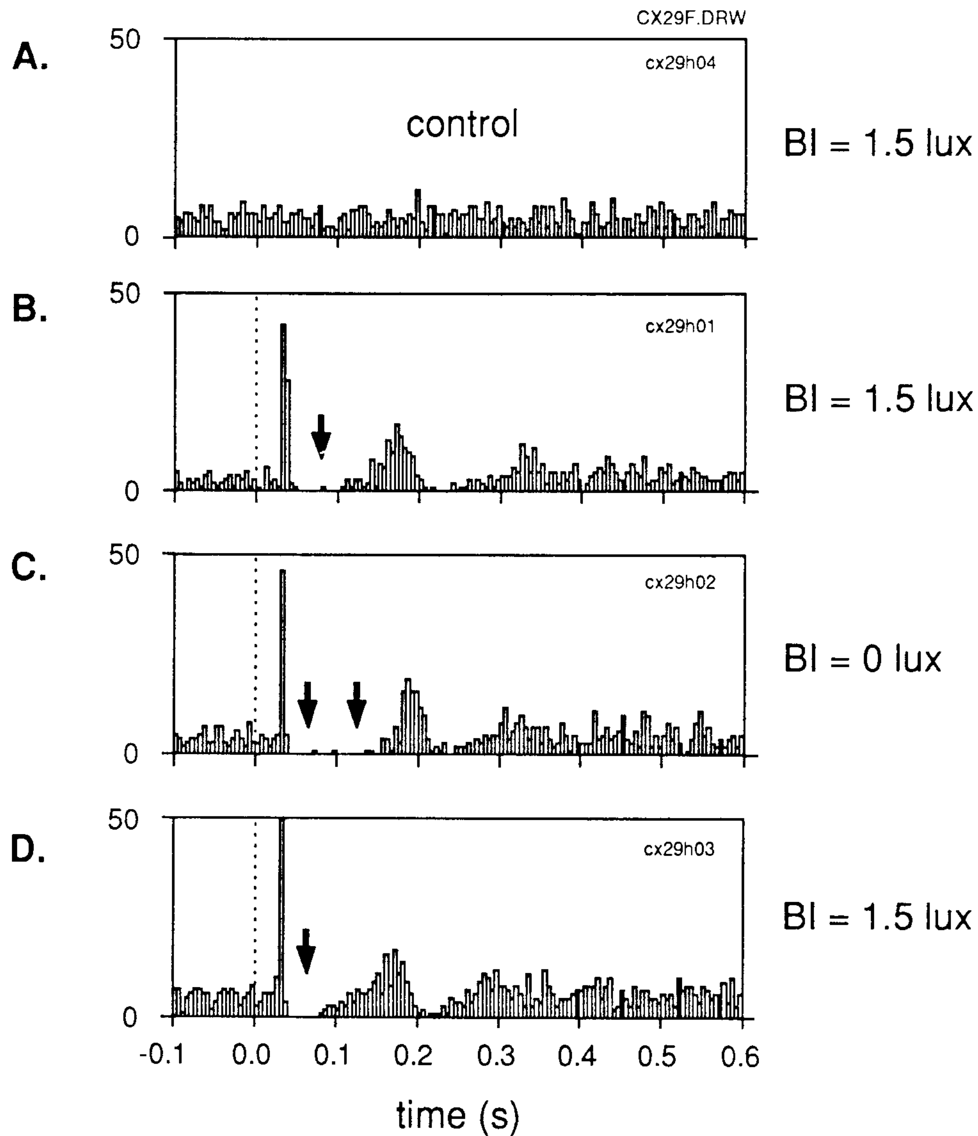


Fig. 5.8. Extracellular responses of a single cortical neurone to flash stimulation under different levels of background illumination (BI). This cell was recorded from an animal receiving constant intravenous (i.v) infusion of equithesin. **A.** Control response - no stimuli applied. **B.** Flash response with background illumination level kept constant at 1.5 lux. Latency to primary excitation = 30 ms, duration of secondary inhibition = 50 ms. **C.** Flash response with background illumination constant at 0 lux i.e. complete darkness. While the latency to the primary excitation is unchanged, the duration of secondary inhibition (arrowed) increased to 110 ms. **D.** Flash response with background illumination back at 1.5 lux. Duration of secondary inhibition decreased to 40 ms. The flash-evoked inhibition was clearly dependent on the level of background illumination.

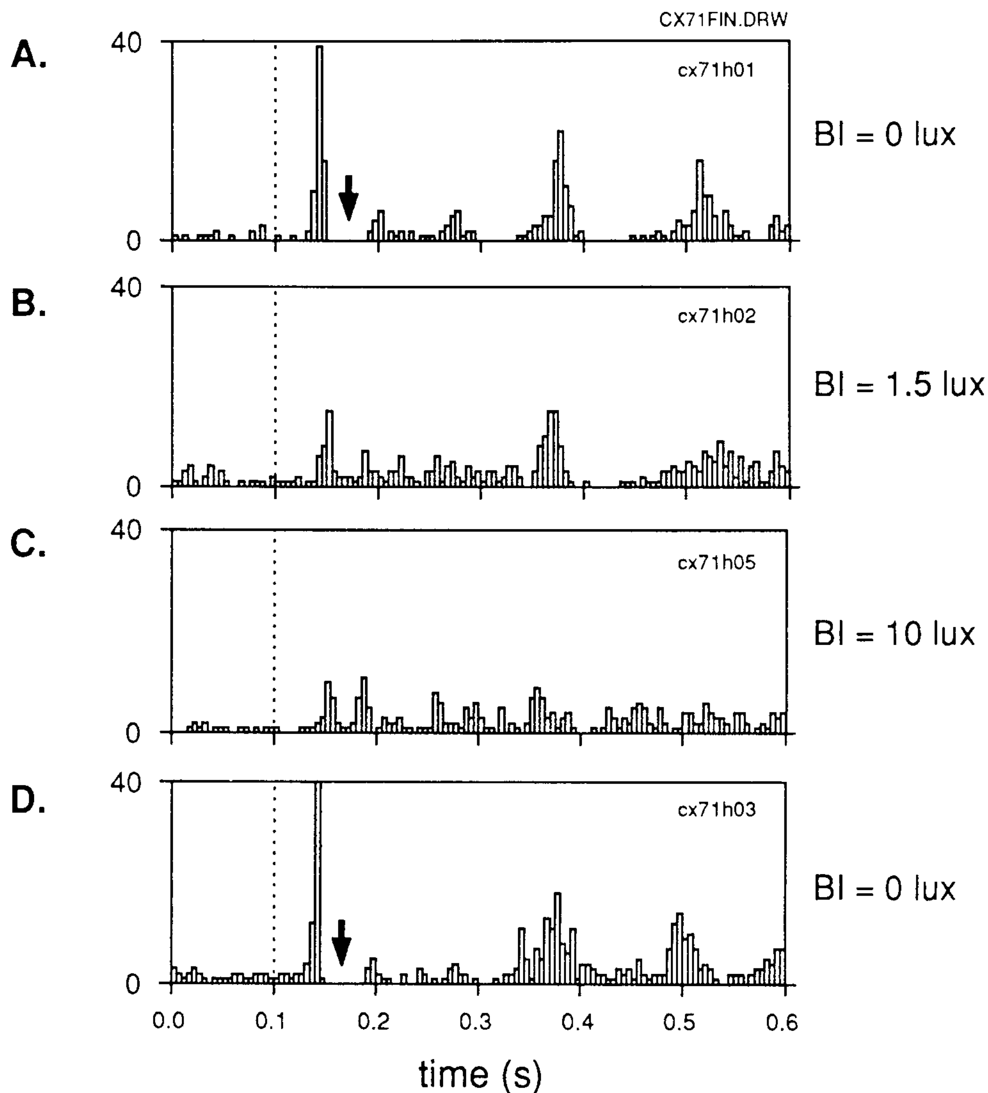


Fig. 5.9. Response of another single cortical neurone to flash stimulation with different levels of background illumination (BI). As in the previous example, this cell was recorded from an animal receiving constant i.v. infusion of equithesin. **A.** Flash response with zero background illumination i.e. complete darkness. Flash stimulation induced a characteristic early excitatory phase after a latency of 38 ms. Thereafter, a 40 ms inhibitory phase emerged (arrowed). Occasional bouts of excitation and inhibition completed the response. **B.** Flash response with background illumination at 1.5 lux. The flash-evoked primary excitatory phase of this cell was markedly attenuated at post-zero levels of background illumination. The brief bout of inhibition (as observed and arrowed in A) disappeared at this level of background illumination. **C.** Further increase in the background illumination to 10 lux maintained the sub-optimal response to flash stimulation. **D.** Return to zero background illumination produced the optimal response, and the re-emergence of the 40 ms bout of inhibition.

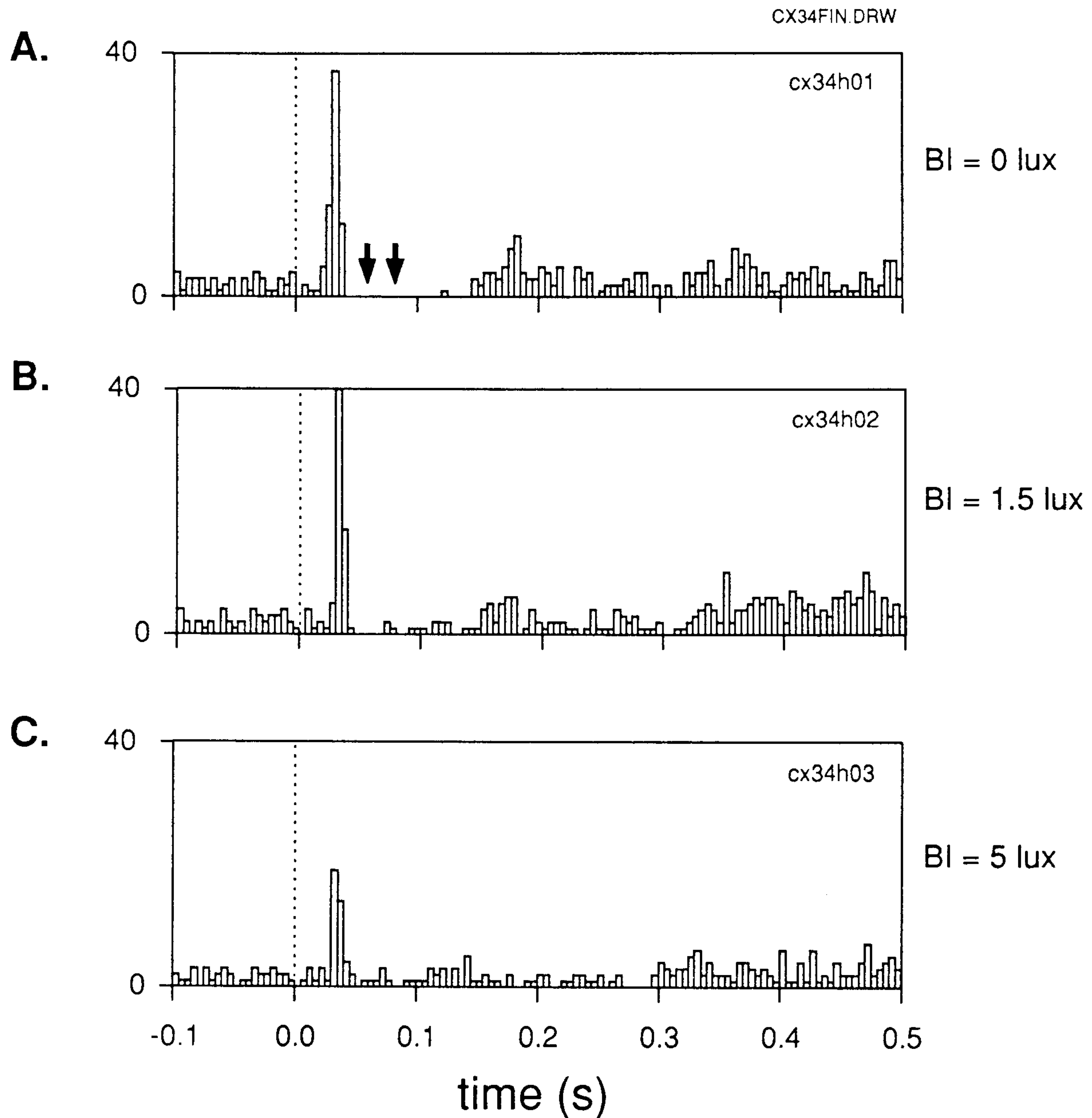


Fig. 5.10. Response of a single cortical neurone to flash stimulation with different levels of background illumination (BI). As in previous examples, this cell was recorded from an animal receiving constant i.v. infusion of equithesin. **A**. Flash response with zero background illumination. Flash stimulation induced a characteristic early excitatory phase after a latency of 30 ms. Thereafter, a prominent inhibitory phase emerged (arrowed). **B**. Flash response with background illumination at 1.5 lux. The flash-evoked primary excitatory phase of this cell was not attenuated at this level of background illumination. However, the period of inhibition (as observed and arrowed in A) did decrease. **C**. An increase in the background illumination to 5 lux now produced a sub-optimal response to flash stimulation i.e. note the smaller magnitude of the early peak.

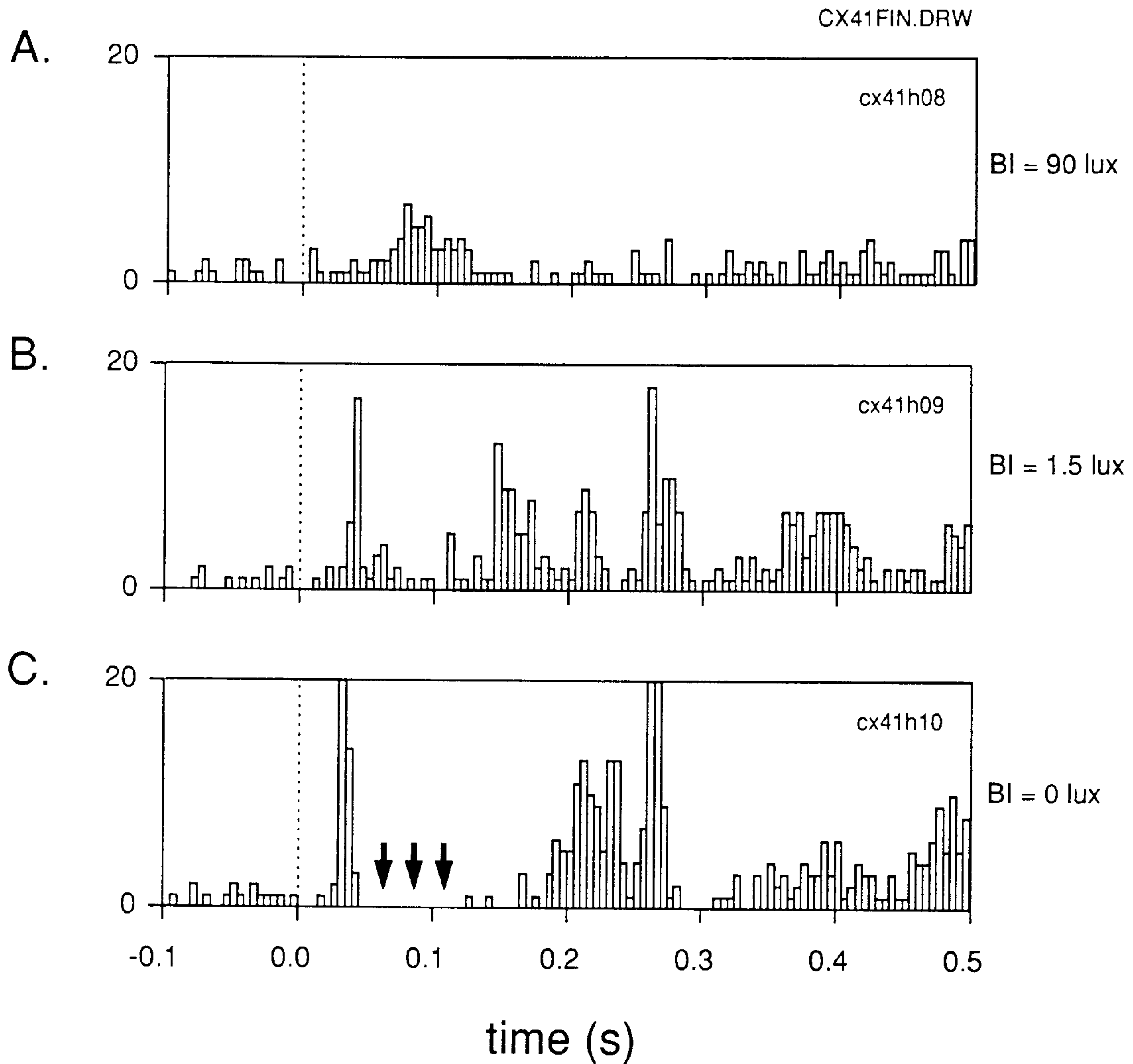


Fig. 5.11. Cortical response to flash stimulation from an animal receiving constant i.v. infusion of equithesin. **A**. Flash stimulation with the lowest contrast produced a weak and sluggish response with poorly defined peaks. **B**. Flash response with background illumination at 1.5 lux. The evoked response was vigorous and showed distinct primary and secondary excitatory peaks. **C**. Flash response with zero background illumination. Once again, the highest contrast produced an early excitatory peak that was followed by a prominent secondary phase of inhibition (arrowed). Some secondary *excitatory* peaks followed this inhibitory phase.

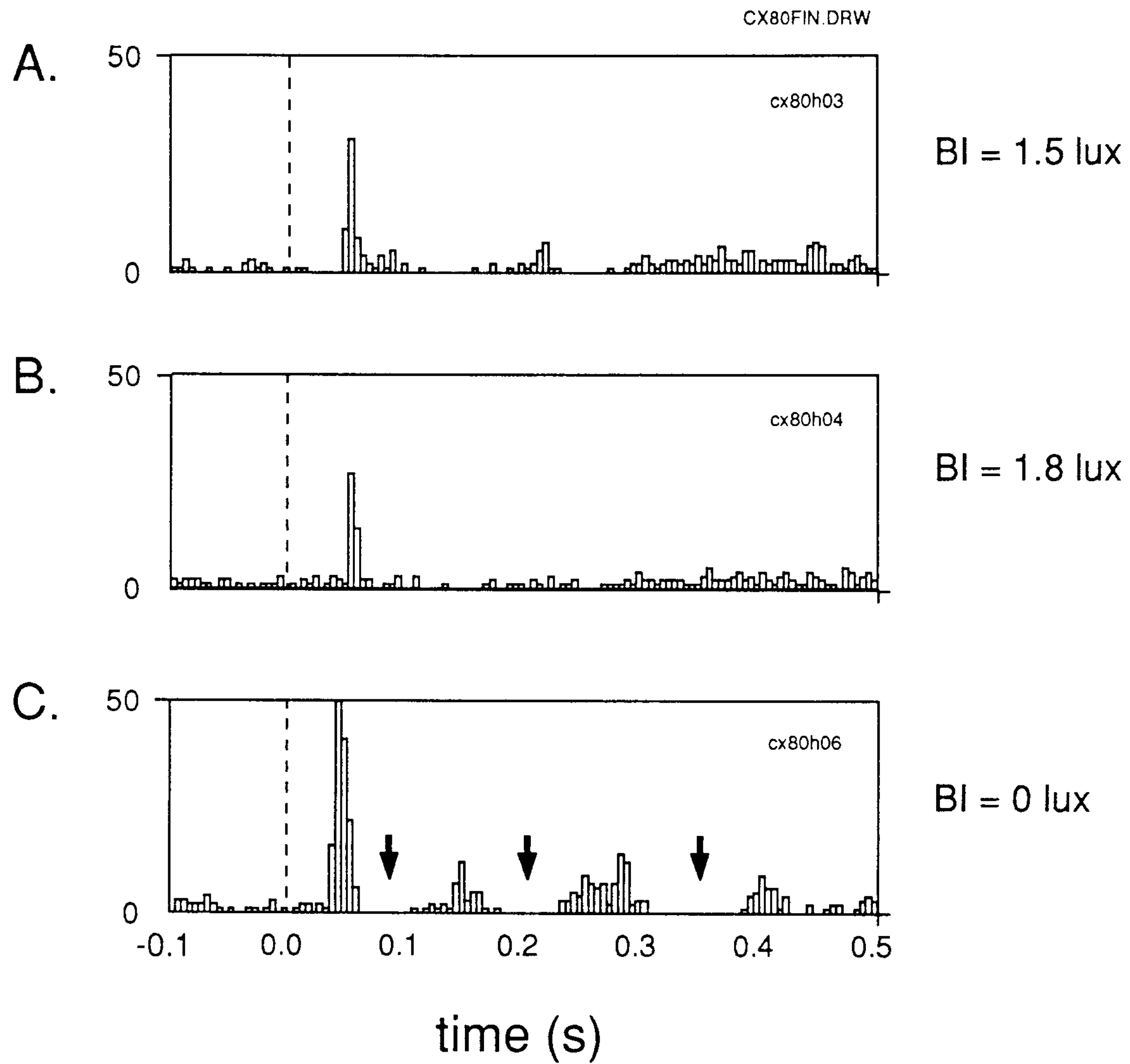


Fig. 5.12. Response of a cortical neurone to flash stimulation recorded from an animal receiving constant i.v. infusion of equithesin. Like previous examples, flash stimulation at 0 lux (highest contrast) produced the largest response. In this example, the prominent early spike was followed by coupled phases of inhibition (arrowed) and excitation that completed the response.

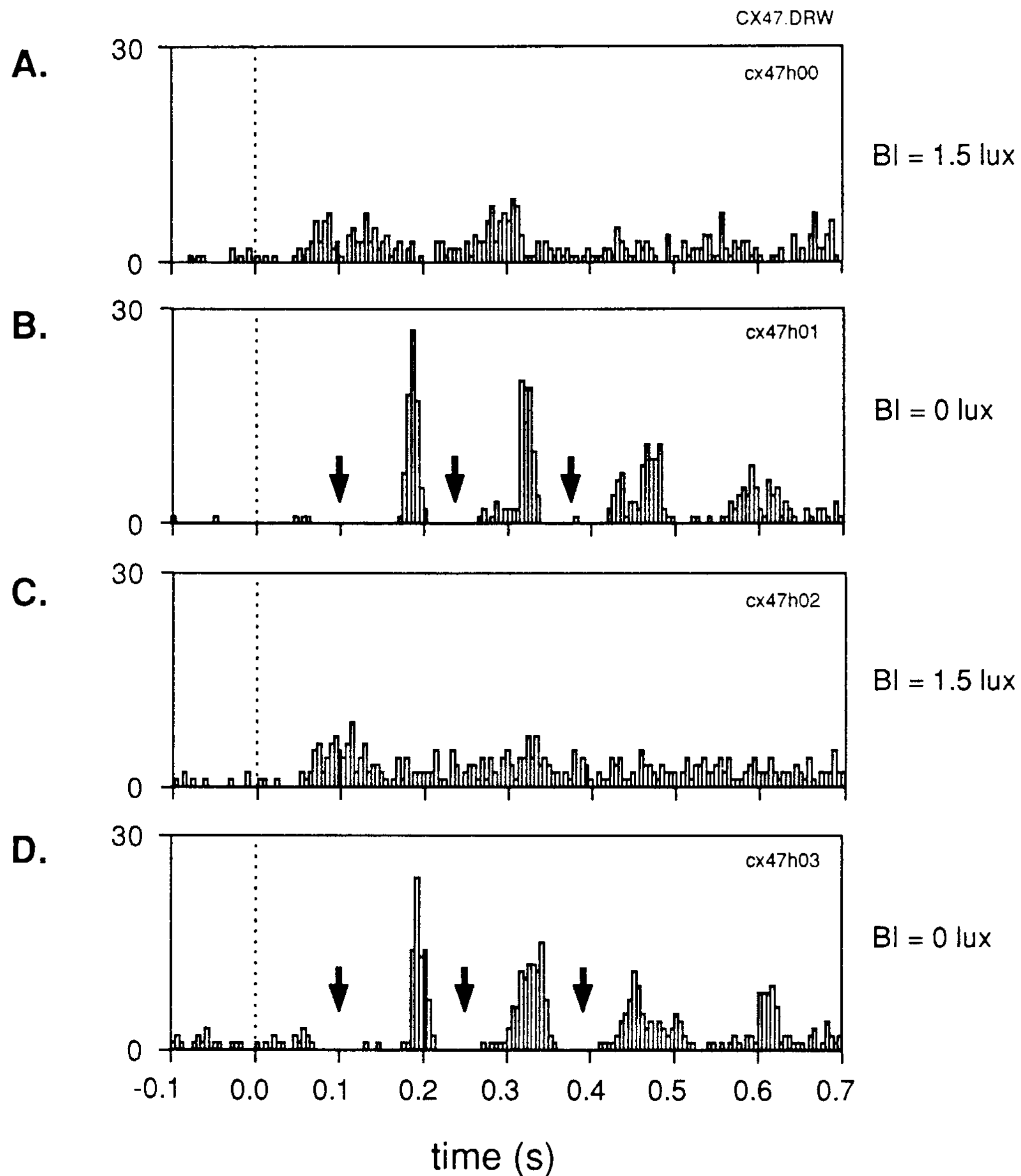


Fig. 5.13. Response of a cortical neurone to flash stimulation with different levels of background illumination (BI). Recorded from an animal receiving constant i.v. infusion of equithesin. Unlike the two previous examples, at zero BI, here flash stimulation produced a *primary* inhibitory phase, followed by repeated bouts of excitation and inhibition. **A**. Flash stimulation with BI at 1.5 lux produced a response with no distinct excitatory peaks. **B**. Flash stimulation with zero BI produced repeated bouts of inhibition (arrowed) coupled with four distinct excitatory phases. This was the only cortical cell where we found a primary inhibitory phase rather than a primary excitatory phase with flash stimulation. **C**. Return to BI of 1.5 lux produced a sub-optimal response. **D**. Once again, flash stimulation with zero BI produced the protracted coupled inhibitory and excitatory response. The repeated recordings of the response of this cortical unit with different levels of background illumination show that this "sensitivity" to the level of background illumination was a function of the level of BI rather than an artefact of fluctuating levels of anaesthesia.

As pointed out in section 5.6 above, the response to electrical and flash pulse stimuli were recorded in 20 visual cortical neurones. The response of some of these neurones have already been presented in section 5.5 (electrical stimulation) and section 5.6 (flash stimulation). It was more convenient to present the cortical response to electrical and flash pulse stimulation separately, because within each of these sections, further complexities were inherent i.e. monopolar vs bipolar electrical stimulation, flash response with different anaesthetics and levels of background illumination.

A single example (from the group of 20) that specifically highlights the difference in the cortical response to flash and electrical pulse stimulation is shown in Fig. 5.14. The first histogram (A) represents the response of the cortical unit to flash stimulation with the lowest level of surround illumination i.e. 0 lux. As evident in previous examples, primary and secondary excitatory phases dominated the response. Two small (20 ms) bouts of inhibition were embedded in the response (filled arrows). In the immediately preceding section, it was shown that upon flash stimulation, the greatest amount of inhibition in any cortical cell that is sensitive to surround illumination, is visible at the lowest level of surround illumination i.e. 0 lux. Similarly, we found that the two 20 ms bouts of flash-evoked inhibition visible in Fig. 5.10 A, was the maximum possible inhibition for this particular cell i.e. post-zero levels of surround illumination produced no inhibitory phases in this neurone. The response of this very same neurone to graded electrical pulse stimulation is displayed in Fig. 5.14 (B-E). Sub-threshold currents were ineffective in eliciting inhibition or excitation (see B in Fig. 5.14). Threshold currents produced moderate amounts of inhibition (see C-D). Optimal currents produced the greatest amount of inhibition, 180 ms in duration (see E). Clearly, such a sustained inhibition never occurred with flash stimulation (see A).

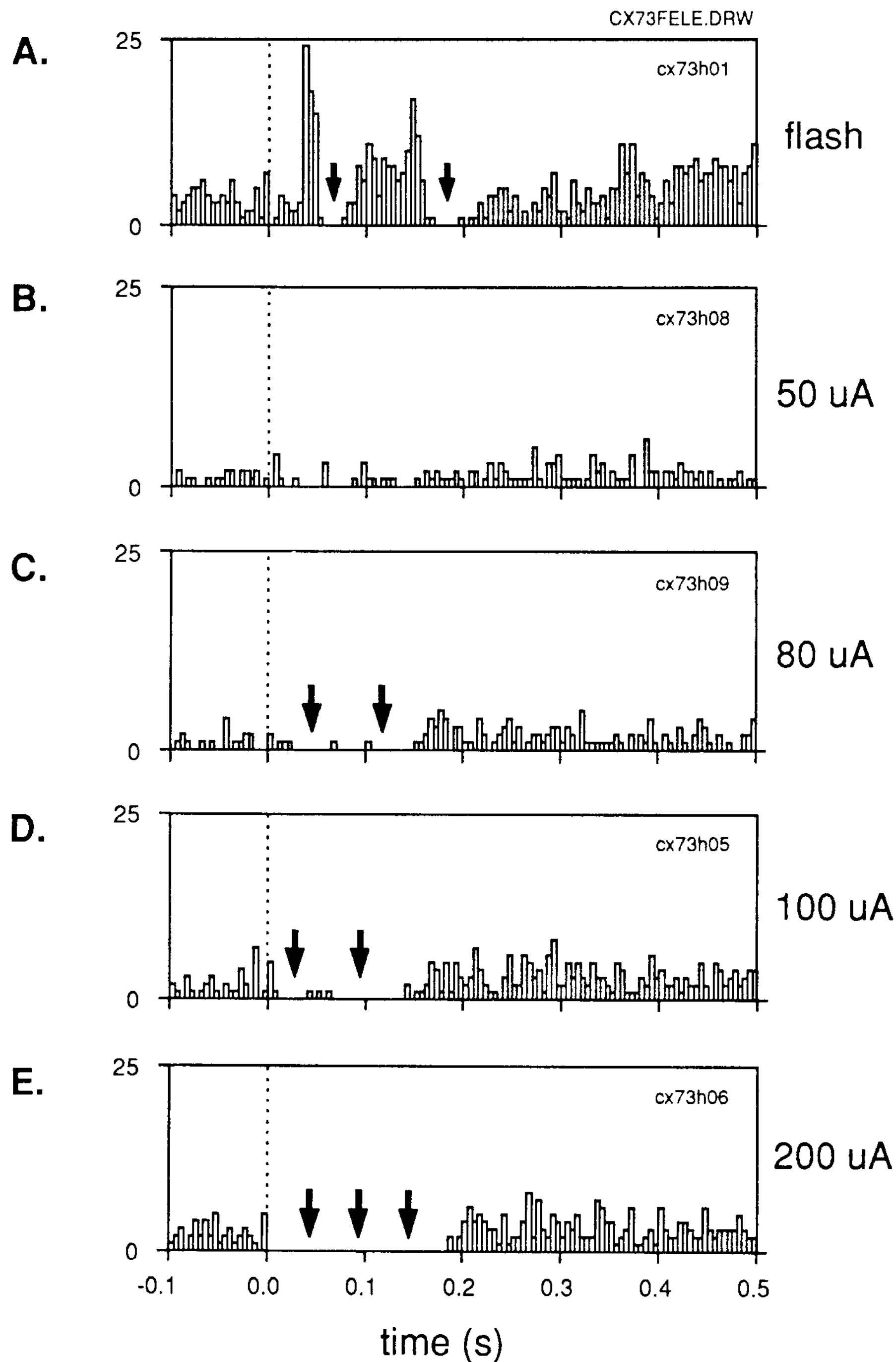


Fig. 5.14. Cortical response to flash stimulation of the contralateral eye (**A**) and subsequent electrical stimulation (**B-E**) of the LGN. Flash response recorded at zero background illumination (BI), where the duration of secondary inhibition was found to be the greatest. Flash-evoked response at post-zero levels of BI not shown. Electrical stimulation at sub-threshold currents e.g. 50 μA (**B**) produces little excitation or inhibition. Threshold currents 80-100 μA (**C-D**), produced some inhibition (arrowed). Optimal electrical stimulation (**E**) produced a sustained inhibitory phase (180 ms). Such protracted inhibitory phases never emerged with flash stimulation, irrespective of the level of background illumination.

5.7. Discussion

5.7.1. *General*

The results show that there is a clear and consistent difference between the response of cortical cells to natural (strobe-flash) and artificial (electrical) pulse (< 1 ms) stimulation.

The response of cortical neurones to electrical stimulation of the ipsilateral dLGN was, in all cases tested, that of a long inhibitory phase (197 ± 61 ms) followed by some rebound (excitatory) activity. With bipolar electrical stimulation, these sustained inhibitory events were often preceded with early ortho- or anti- dromic spikes. Flash stimulation (of the contralateral eye) however, produced primary and secondary excitatory events after a latency of 30-60 ms from the application of the stimulus, in all of the tested units, irrespective of the type of anaesthetic used. These events extended to 200-300 ms. No protracted level of inhibition was observed either before or after these excitatory events.

The temporal profile of the cortical response to flash stimulation could often be modulated by small changes in the intensity of the surround (or background) illumination. Most evident, was the emergence of a secondary inhibitory phase at the lowest level of background illumination i.e. in complete darkness (0 lux). Just one cortical unit displayed a *primary* inhibitory phase at the lowest level of surround illumination.

A major finding is that both electrical and flash pulse stimuli elicit inhibition in the visual cortex. The difference in the response is that flash-evoked inhibition was always much shorter than electrically-evoked inhibition, provided that the visual units were recorded at the most optimal level of background illumination (for flash stimulation) or with the most optimal stimulus strength (for electrical stimulation). Further, the response to electrical stimulation shows a very "switching" profile i.e. first ortho- or anti-dromic spike, then protracted inhibition, and finally rebound excitation. With flash stimulation, excitation and inhibition are tightly coupled, but excitation almost always overrides inhibition.

5.7.2. *Comparison of the present data with previous studies*

Surprisingly, no studies have compared the response of visual cortical neurones to transient electrical and flash stimulation. Many studies have recorded the response of cortical neurones to a multitude of different forms of natural stimuli e.g. moving bars (Burne *et al*, 1984; Douglas *et al*, 1991) checker-like blocks (Richmond *et al*, 1990), and even faces (Perrett *et al*, 1987). Using these types of stimuli (in conjunction with standard electrophysiological techniques) in an attempt to unravel the functional circuitry of the visual cortex, has met with poor success (see Martin, 1988a; Berman *et al*, 1992; Chapter 3; for reviews). This may have been as a result of these natural forms of stimuli being applied for periods that are very much longer than the duration of synaptic events, and so, the details of intracortical processing were obscured. By contrast, the stimuli used in this study were much shorter than the duration of synaptic events, and therefore acted as a suitable probe for analysing the mechanism of intracortical processing (see chapter 1 & Introduction to this chapter).

A few studies (Creutzfeldt *et al*, 1969; Kunt & Creutzfeldt, 1971) have recorded the response of visual cortical neurones to flash pulse stimulation only. Separate studies (Douglas *et al*, 1989; Douglas & Martin, 1991) have recorded the response of cortical neurones to electrical pulse stimuli. These have however, been confined to intracellular cat studies. In the remainder of this section, we compare (where possible) the temporal pattern of the response from these cat studies to our extracellular data obtained from rats.

Cortical response to electrical stimulation

Douglas *et al* (1989) and Douglas & Martin (1991) have recorded the intracellular response of cat visual cortical neurones (*in vivo*) to 0.2-0.4 ms electrical pulse stimuli. Their findings have been discussed in detail in chapter 3. The common feature of our extracellular and their intracellular responses to electrical pulse stimulation was the presence of a protracted inhibitory phase, 100-300 ms in duration. Douglas & Martin (1991) showed that this inhibition was largely hyperpolarising rather than shunting. Extracellular recordings clearly cannot reveal the degree of hyperpolarising or shunting inhibition. The intracellular method is technically difficult (see Douglas *et al*, 1991) and has never been successfully performed in *in vivo* rat studies, irrespective of the types of stimuli used. Intracellular recordings in

rats have been successfully performed only in *in vitro* slice preparations (Berman *et al*, 1989; Berman, 1991). Even in the *in vivo* cat preparation, there have been only a few studies (Creutzfeldt *et al*, 1969; Douglas *et al*, 1991, Douglas & Martin, 1991) that have successfully used the intracellular method for analysing cortical processing (see Martin, 1988a; for a review).

Cortical response to flash stimulation

Creutzfeldt *et al* (1969) and Kunt & Creutzfeldt (1971) recorded the response of striate (i.e. primary visual) cortical neurones to strobe-flash (10 μs duration) stimulation . Unlike our study, they used cats as the experimental animal and the recordings were intracellular. Their findings have been reviewed in chapter 3.

Creutzfeldt *et al* (1969) grouped their cortical responses into two distinct classes. The first group of cortical cells (class 1) responded with primary and secondary excitatory phases, similar to those observed in this study (e.g see Fig. 5.4). The average latency to primary excitation (in Creutzfeldt *et al*'s study) was found to be 50 ms. The neuronal activity in the test period was always higher than that of the control (at least for the first 200-300 ms). These findings are consistent with the data obtained in our study. The second group of cortical cells (class 2) responded with a primary inhibitory phase that was either sustained or was followed by excitation. This group (class 2) represented 66% of their sample while the first group (class 1) accounted for just 34%. These findings go against our data as the vast majority of visual cortical neurones recorded in our study responded with a primary excitatory phase instead of a primary inhibitory one. Only 1 of the 20 tested units i.e. 5% (where the surround illumination was tightly controlled and adjusted for optimality) responded with primary inhibition (Fig 5.13). There is overwhelming microanatomical evidence (see chapter 3) which show that approximately 80% of synaptic contacts on spiny pyramidal neurones are excitatory. Furthermore, it has been established that the spiny (excitatory) neurones in cortex (in the rat) account for 85-90% of the cortical population (see chapter 3). So the probability of encountering a smooth (inhibitory) neurone is relatively small. Consequently, it is possible that the single cortical cell that displayed a primary inhibitory phase (Fig. 5.13) was a smooth cell. Creutzfeldt *et al*'s finding that 66% of their recorded sample of cortical neurones responded with a primary inhibitory phase is most baffling. Even in cats, the spiny cells account for at least 66% of the total neuronal

population (see chapter 3). It is expected that spiny cortical neurones, in response to natural stimuli, respond with a primary excitatory phase, provided the appropriate receptive fields are being stimulated. This is because the thalamic afferents make only excitatory contacts with their cortical targets (Dehay *et al*, 1991). So, it is possible that Creutzfeldt *et al* did not position the flash unit in the appropriate receptive fields of individual cortical neurones. Furthermore, Creutzfeldt *et al* presented their PSTH results as the summed response of *all* the cells of a particular class. This most likely masked the true excitatory and/or inhibitory phases of individual cells. In our study, the response of individual cells were always analysed and presented separately, accurately revealing excitatory or inhibitory phases of tens to hundreds of milliseconds in duration.

It should be noted that the class 1 cells of Creutzfeldt *et al* i.e. those that responded with primary excitation were, like the cortical units recorded in this study, also sensitive to the level of background illumination. However, in contrast to our study (where the duration of secondary inhibition was highest without background illumination i.e. 0 lux), they found that the duration of inhibition was highest *with* background illumination (10 lux). These differences might be species related and further discussion on this is beyond the scope of this study. Our objectives in this study were not to assess the sensitivity of the cortical response to flash stimulation to different levels of surround illumination, and then compare them to other species. The reason for recording the flash response with different levels of background illuminations was to ensure that the sustained excitatory component (upon flash stimulation) was largely independent of the level of background illumination. While the majority of cortical neurones were indeed sensitive to the level of the background illumination, that sensitivity merely represented a 40-70 ms increase in a secondary inhibitory phase. Changing the level of surround illumination does not remove the flash evoked re-excitation that is such a common feature in the visual cortex. Similarly, it is not implied that the overall responsiveness of the cell is unaffected by the level of background illumination. Indeed, the majority of visual cortical neurones recorded in this study responded best at subdued levels of background illumination (0-10 lux). This might be expected as rats are primarily nocturnal (Sefton & Dreher, 1985).

Douglas *et al* (1989) found that the geniculate (thalamic) input to layers 2, 3 (and 4) is larger than that of layers 5 and 6. Thus, it may be that many of the cells in layer 5 and 6 receive a larger intracortical input as compared to the

superficial layers. Consequently, if the geniculate input to the deep layers alone cannot cause them to depolarise past threshold then their excitation will largely depend on the superficial pyramidal cells firing first. In such instances, the latency to initial excitation of the deeper cortical cells would be expected to be longer than the superficial ones. Our cortical recordings being extracellular, do not allow for direct identification of cell types and their corresponding depths in striate (i.e. primary visual) cortex. Our histological records allow us to identify only the area of recording e.g. whether the penetrations were in striate cortex or not. So the broad range of latencies (30-60) to primary excitation (upon flash stimulation) might depend on the depth of the recording electrode in cortex. Such detail was of little value to the overall objectives of this study, and so was not pursued any further.

The above responses of striate cortical cells to electrical and especially flash pulse stimulation cast considerable doubt on traditional models of:
(a) orientation selectivity e.g. Hubel & Wiesel (1962) and Ferster (1988); and
(b) various other perceptual tasks that were thought to rely predominantly on rapid serial processing (as in conventional digital systems).
This is because the secondary excitatory peaks and rebounds (upon flash stimulation) and prolonged inhibition (with electrical stimulation) that extend to a few hundred milliseconds (even though the stimuli are less than a millisecond in duration) are characteristic of a "mass action" behaviour. These findings provide the first threads of experimental i.e. electrophysiological evidence that support current beliefs of extensive parallel processing in the neocortex (Crick, 1989; Sefton & Dreher, 1985; Martin, 1988 a & b; Douglas & Martin, 1991). The electrical stimulation responses however, are less convincing as it is very unlikely that such synchronous (non-specific) activation of fibres (Ranck, 1981) would occur with any form of natural stimulation. The acceptability of models based on artificial stimulation are thus questioned. A "mass-action" phenomenon manifesting itself as cortical re-excitation (as that seen upon flash stimulation) is very plausible considering the overwhelming amount of microanatomical evidence (chapter 3) which show that cortical neurones receive mainly excitatory contacts, predominantly from other cortical neurones. The LGN (or thalamic input in general) accounts for approximately 20% of the synapses on cortical neurones, and these are all excitatory (see chapter 3). However, since it is the LGN (situated in the thalamus) which provides the visual cortex with incoming retinal information, it is possible that this flash-evoked cortical re-excitation might still originate from the LGN itself. Consequently, the next chapter of this thesis focuses on the flash response of LGN (thalamic) neurones.

Chapter 6

RESULTS & DISCUSSION - PART 2

THALAMIC RESPONSE TO TRANSIENT STIMULI

6.1. Introduction

This chapter focuses specifically on the response of dorsolateral geniculate nucleus (dLGN) neurones to strobe-flash stimulation of the contralateral eye. The motivation for this series of experiments stems directly from the previous chapter, where the striate (or primary visual) cortical response to strobe-flash stimulation was recorded. There, it was shown that cortical neurones responded to flash stimuli in a "compound" way i.e. apart from a prominent primary excitatory phase, secondary excitatory events extending to hundreds of milliseconds were the order. By evaluating the response of dLGN neurones to the same type of transient stimulus (< 1 ms in duration), we could determine whether the LGN played a role in contributing to the compound cortical response, especially that of the secondary phase of re-excitation. In this instance, it is not expected that the LGN response to flash stimuli be of an identical profile to that of striate cortical cells, because each of these areas have quite different synaptic organisations (chapters 2 & 3). It could be argued that if LGN neurones were to display a sustained excitatory phase, then this would provide good circumstantial evidence that the cortical phase of re-excitation could be directly dependent on the thalamic input. On the other hand, the absence of sustained excitatory events in LGN neurones (with flash stimulation) would support hypotheses and models that rely on *intracortical* excitation (and inhibition) to mould the response of striate cortical neurones to natural stimuli.

6.2. Methods for Optimal Positioning of the LGN Recording Electrodes

The criteria used for LGN penetrations here are similar to those used for the insertion of stimulating electrodes in the LGN (see chapter 5, section 5.3). Briefly, all LGN penetrations were approached in the vertical plane using glass fibre-filled recording microelectrodes (see chapter 4, section 4.3). Starting penetrations were usually aimed at 3.8 mm posterior Bregma and

3.5 mm lateral to midline. Thereafter, penetrations were made at 0.3 mm to the starting penetration either in the anterior-posterior or lateral plane. The small size of the dLGN (±1.6x1.0x0.8 mm^3 - calculated from the atlas of Paxinos & Watson, 1986) restricted the number of penetrations made per animal, if I was to avoid damaging the LGN. Usually, just three penetrations were made in each animal. Each penetration started at the surface of cortex (non-striate). From the surface to approximately 3.5 mm deep, the coarse microdrive manipulator was used. From then onwards, the fine micro-manipulator (2 µm vertical resolution) was used. This was because the stimulating electrode penetrations (see chapter 5) in the dLGN (and the atlas of Paxinos & Watson, 1986) suggested that the optimal depth for obtaining visual responses was between 3.8 and 4.4 mm from the surface of cortex. It was found that the most probable depth of recording single units in the dLGN to be at 4.2 mm from the surface of cortex (range: 3.8 - 4.8 mm, see Table 6.2).

The pattern of activity as the recording electrodes were advanced towards the dLGN is similar to that described for the approach with the stimulating electrodes (see chapter 5). However, while the LGN penetrations using the tungsten stimulating electrodes never yielded single unit activity, the glass electrodes did. This is most likely attributed to the configuration of the glass recording electrode i.e. its smaller tip diameter as compared to the tungsten stimulating electrode (see chapter 4).

6.3. General Characteristics and Observations

A total of 55 LGN (or thalamic relay) units from 12 rats were considered suitable for this study. Only 6 (11%) of these units were found to be completely unresponsive to hand-held spot or strobe-flash (i.e. natural) stimuli. The remaining 49 (89%) could be classified as either of the "ON type" i.e. excited by visual (hand-held spot or strobe-flash) stimuli, or the "OFF type" i.e. inhibited by visual stimuli (see Tables 6.1 & 6.2). All LGN units were recorded from rats anaesthetised with either intraperitoneal (i.p) or continuous intravenous (i.v) equithesin (see Table 6.2 for further details).

Like the cortical recordings (chapter 5), no attempt was made to quantify the detailed receptive field structures of the recorded sample of thalamic neurones. However, using spot stimuli, LGN relay cells appeared to have concentric ON or OFF like receptive fields. Most certainly, the size of the

receptive fields of LGN neurones were considerably smaller than those of visual cortical neurones.

Table 6.1: Population of recorded LGN neurones

	(n)	(%)
ON-like	37	67
OFF-like	12	22
NON visual	6	11
Total	55	100

Note: 7 of these ON and 1 OFF like units were lost during the logging procedure. See Table 6.2 for complete data set.

6.4. Response to Strobe-Flash Stimulation

The temporal response to flash pulse stimulation was recorded in 30 of the 37 ON-like and 11 of the 12 OFF like LGN neurones (see Tables 6.1 & 6.2). The latency of the ON- and OFF-like LGN responses for the recorded population is summarized in Fig. 6.0.

Four typical responses of ON-like LGN neurones to flash pulse stimulation are shown in Fig. 6.1 (A-D). Additional examples presented in Fig. 6.2 - 6.5 will be discussed later. The animals used to record the extracellular response of cells A & B (in Fig. 6.1) were anaesthetised with equithesin administered intraperitoneally (i.p.). The extracellular response of cells C & D were recorded from animals anaesthetised with equithesin administered intravenously (i.v.). Flash stimulation produced a pronounced (but brief) excitatory phase (8-16 ms duration) after a latency of 30-40 ms. This was followed by a sustained inhibitory phase lasting 250-400 ms. 21 of the 30 (70%) tested units showed a late excitatory discharge (LD) that interrupted or followed the sustained inhibition (e.g. see Fig. 6.1 B, arrowed). This late discharge (LD) or rebound excitatory activity is unlikely to contribute to the secondary excitatory activity seen in striate cortical neurones upon flash stimulation (see chapter 5). This is because much of the secondary phase of cortical re-excitation is already initiated and propagated (for 200-300 ms) before the LGN neurones fire their LDs (after 250-300 ms of inhibition). Whether this holds true in the face of changing levels of background illumination is an important aspect of this chapter and will be considered below.

Table 6.2: LGN single flash response data
Key:

REF: Reference number used to log the cell's response under a specific test condition.

CN: Cell number. Only visual units that were logged are shown.

RN: Rat or experiment number.

GR: Arbitrary grade of response.

ANAES: Type of anaesthesia. i.p. equi - intraperitoneal injection of equithesin, i.v. equi - intravenous administration of equithesin.

ILLUM: Level of surround or background illumination (measured in lux).

DEPTH: Depth of the recording electrode in the LGN.

SACP: Spontaneous activity in the control period.

ON/OFF: whether the logged units were switched ON (excited) or OFF (inhibited) upon visual stimulation.

LD: whether the units displayed any late (or secondary) excitatory discharges.

HIST: whether the electrode tracts could be confirmed to be in the target area by histological examination of coronal sections.

NA: not applicable e.g. unable to discriminate peak because of sub-optimal level of background illumination.

	A	B	C	D	E	F	G	H	I	J	K	L
1	REF	CN	RN	GR	ANAES	ILLUM	DEPTH	SACP	on/off	LAR	LD	HIST
2	LR1K43	L1	43	4	i.p. equi	2.5	4	y	ON	39	n	no
3	LR3K43	L2	43	4	i.p. equi	2.5	4.02	y	ON	39	n	no
4	LR4K43	L3	43	5	i.p. equi	2.5		little	ON	35	n	no
5	LR6K43	L4	43	3	i.p. equi	2.5	4.36	y	ON	40	y	no
6	LR7K43	L5	43	5	i.p. equi	2.5	3.82	y	ON	33	y	no
7	LR8K43	L6	43	4	i.p. equi	2.5	4	y	ON	38	y	no
8	LR9K43	L7	43	4	i.p. equi	2.5	4.24	y	ON	43	y	no
9	LR10K43	L8	43	4	i.p. equi	2.5	4.37	y	ON	43	y	no
10	LR11K43	L9	43	4	i.p. equi	2.5	4.5	y	ON	40	y	no
11	LR12K43	L10	43	4	i.p. equi	2.5	4.61	y	ON	40	y	no
12	LR13K43	L11	43	4	i.p. equi	2.5	4.38	y	ON	43	n	no
13	LR17K43	L12	43	4	i.p. equi	2.5	4.15	y	ON	38	n	no
14	LR22K43	L13	43	5	i.p. equi	2.5	4.25	y	ON	36	y	no
15	LR23K43	L14	43	5	i.p. equi	2.5	4.45	y	ON	38	y	no
16	LR18K43	L15	43	5	i.p. equi	2.5	3.9	y	ON	36	y	no
17	LR19K43	L15	43	5	i.p. equi	30	3.9	y	ON	43	y	no
18	LR20K43	L15	43	5	i.p. equi	2.5	3.9	y	ON	36	y	no
19	LR21K43	L15	43	5	i.p. equi	30	3.9	y	ON	43	y	no
20	LR24K43	L16	43	5	i.p. equi	2.5	4.05	y	ON	37	n	no
21	LR29K43	L17	43	5	i.p. equi	2.5	4.05	n	ON	40	y	no
22												
23	lgn11h00	L18	119	3	i.v. equi	1.5	4.8	little	ON	50	n	no
24	lgn11h01	L18	119	3	i.v. equi	0	4.8	little	ON	50	n	no
25	lgn11h09	L18	119	3	i.v. equi	0	4.8	little	ON	50	n	no
26	lgn11h10	L18	119	3	i.v. equi	1.5	4.8	little	ON	55	n	no
27	lgn11h11	L18	119	3	i.v. equi	5	4.8	little	ON	55	n	no
28												
29	lgn15h00	L19	125	3	i.v. equi	1.5	4.12	n	OFF	NA	y	yes
30	lgn15h01	L19	125	3	i.v. equi	0	4.12	n	OFF	NA	y	yes
31	lgn15h02	L19	125	3	i.v. equi	90	4.12	n	OFF	NA	n	yes
32	lgn15h03	L19	125	3	i.v. equi	1.5	4.12	n	OFF	NA	y	yes
33												
34	lgn17h00	L20	125	1	i.v. equi	1.5	4.65	little	ON	50	n	yes
35	lgn17h01	L20	125	1	i.v. equi	1.5	4.65	n	ON	50	n	yes
36	lgn17h02	L20	125	2	i.v. equi	0	4.65	little	ON	50	y	yes
37	lgn17h03	L20	125	2	i.v. equi	1.5	4.65	little	ON	50	n	yes
38	lgn17h04	L20	125	2	i.v. equi	5	4.65	y	ON	45	n	yes
39	lgn17h05	L20	125	4	i.v. equi	0	4.65	y	ON	50	n	yes
40	lgn17h06	L20	125	3	i.v. equi	1.5	4.65	y	ON	50	n	yes
41	lgn17h07	L20	125	1	i.v. equi	90	4.65	y	ON	NA	n	yes
42	lgn17h08	L20	125	3	i.v. equi	1.5	4.65	y	ON	50	y	yes
43	lgn17h09	L20	125	4	i.v. equi	0	4.65	y	ON	50	n	yes
44												
45	lgn22h00	L21	131	5	i.p. equi	1.5	4.01	little	ON	25	y	yes
46	lgn22h01	L21	131	5	i.p. equi	0	4.01	little	ON	25	y	yes
47	lgn22h02	L21	131	5	i.p. equi	1.5	4.01	little	ON	30	y	yes
48	lgn22h03	L21	131	5	i.p. equi	1.5	4.01	y	ON	35	y	yes
49	lgn22h04	L21	131	4	i.p. equi	0	4.01	y	ON	35	y	yes
50	lgn22h05	L21	131	4	i.p. equi		4.01	y	ON	40	y	yes

	A	B	C	D	E	F	G	H	I	J	K	L
51	lgn22h06	L21	131	1	i.p. equi	5	4.01	y	ON	30	y	yes
52	lgn22h07	L21	131	5	i.p. equi	0	4.01	y	ON	30	y	yes
53	lgn22h09	L21	131	5	i.p. equi	1.5	4.01	y	ON	45	y	yes
54												
55	lgn23h00	L22	131	2	i.p equi	1.5	3.9	n	OFF	NA	y	yes
56	lgn23h01	L22	131	2	i.p. equi	0	3.9	n	OFF	NA	y	yes
57												
58	lgn24h00	L23	131	5	i.p. equi	1.5	4.2	n	ON	40	n	yes
59	lgn24h01	L23	131	5	i.p. equi	0	4.2	n	ON	40	n	yes
60	lgn24h02	L23	131	5	i.p. equi	1.5	4.2	little	ON	40	n	yes
61	lgn24h03	L23	131	4	i.p. equi	5	4.2	little	ON	40	n	yes
62	lgn24h04	L23	131	5	i.p. equi	10	4.2	little	ON	40	n	yes
63	lgn24h05	L23	131	5	i.p. equi	90	4.2	little	ON	35	n	yes
64	lgn24h06	L23	131	5	i.p. equi	0	4.2	n	ON	40	n	yes
65												
66	lgn25h00	L25	132	3	i.v. equi	1.5	4.07	n	OFF	NA	y	yes
67	lgn25h01	L25	132	3	i.v. equi	0	4.07	n	OFF	NA	y	yes
68	lgn25h04	L25	132	3	i.v. equi	1.5	4.07	little	OFF	NA	y	yes
69	lgn25h06	L25	132	2	i.v. equi	90	4.07	n	OFF	NA	n	yes
70												
71	lgn26h00	L26	132	2	i.v. equi	1.5	4.19	n	OFF	NA	y	yes
72	lgn26h01	L26	132	3	i.v. equi	0	4.19	n	OFF	NA	y	yes
73												
74	lgn27h00	L27	132	5	i.v. equi	1.5	4.84	y	OFF	NA	y	yes
75	lgn27h01	L27	132	5	i.v. equi	0	4.84	y	OFF	NA	y	yes
76												
77	lgn30h01	L28	134	2	i.v. equi	0	4.21	y	ON	45	n	yes
78	lgn30h02	L28	134	2	i.v. equi	1.5	4.21	y	ON	45	n	yes
79	lgn30h04	L28	134	3	i.v. equi	1.5	4.21	y	ON	35	y	yes
80	lgn30h06	L28	134	5	i.v. equi	0	4.21	y	ON	35	n	yes
81	lgn30h07	L28	134	5	i.v. equi	5	4.21	y	ON	40	y	yes
82	lgn30h08	L28	134	5	i.v. equi	10	4.21	y	ON	45	y	yes
83	lgn30h09	L28	134	1	i.v. equi	90	4.21	y	ON	NA	y	yes
84												
85	lgn32h00	L29	134	2	i.v. equi	1.5	3.9	little	ON	55	y	yes
86	lgn32h01	L29	134	4	i.v. equi	0	3.9	y	ON	55	n	yes
87	lgn32h02	L29	134	3	i.v. equi	1.5	3.9	little	ON	45	y	yes
88	lgn32h03	L29	134	2	i.v. equi	5	3.9	little	ON	45	y	yes
89	lgn32h04	L29	134	5	i.v. equi	10	3.9	little	ON	45	y	yes
90	lgn32h05	L29	134	3	i.v. equi	90	3.9	n	ON	55	n	yes
91	lgn32h07	L29	134	3	i.v. equi	0	3.9	n	ON	55	n	yes
92												
93	lgn39h00	L31	135	1	i.v. equi	1.5	3.86	little	ON	NA	y	yes
94	lgn39h01	L31	135	5	i.v. equi	0	3.86	little	ON	35	n	yes
95	lgn39h02	L31	135	1	i.v. equi	1.5	3.86	little	ON	45	y	yes
96												
97	lgn41h00	L32	135	5	i.v. equi	1.5	4.73	y	ON	30	n	yes
98	lgn41h01	L32	135	5	i.v. equi	0	4.73	y	ON	30	n	yes
99	lgn41h02	L32	135	5	i.v. equi	5	4.73	y	ON	30	n	yes
100	lgn41h03	L32	135	2	i.v. equi	10	4.73	y	ON	NA	n	yes

	A	B	C	D	E	F	G	H	I	J	K	L
101	lgn41h04	L32	135	1	i.v. equi	90	4.73	y	ON	NA	y	yes
102	lgn41h05	L32	135	5	i.v. equi	0	4.73	y	ON	30	y	yes
103												
104	lgn47h00	L34	138	5	i.v. equi	1.5	4.1	y	OFF	27	y	no
105	lgn47h01	L34	138	5	i.v. equi	0	4.1	y	OFF	27	n	no
106	lgn47h03	L34	138	5	i.v. equi	1.5	4.1	y	OFF	27	n	no
107	lgn47h04	L34	138	5	i.v. equi	5	4.1	y	OFF	27	n	no
108	lgn47h05	L34	138	5	i.v. equi	10	4.1	y	OFF	NA	n	no
109												
110	lgn53h00	L37	140	5	i.v. equi	0	4.4	n	ON	20	y	no
111	lgn53h01	L37	140	5	i.v. equi	1.5	4.4	n	ON	20	y	no
112	lgn53h02	L37	140	5	i.v. equi	10	4.4	n	ON	20	y	no
113	lgn53h03	L37	140	5	i.v. equi	90	4.4	n	ON	20	y	no
114	lgn53h04	L37	140	5	i.v. equi	0	4.4	n	ON	20	y	no
115	lgn53h05	L37	140	5	i.v. equi	1.5	4.4	n	ON	20	y	no
116												
117	lgn54h00	L38	140	5	i.v. equi	0	4.49	n	ON	30	y	no
118	lgn54h01	L38	140	5	i.v. equi	1.5	4.49	n	ON	25	y	no
119	lgn54h02	L38	140	5	i.v. equi	10	4.49	n	ON	30	y	no
120	lgn54h03	L38	140	5	i.v. equi	90	4.49	n	ON	30	y	no
121												
122	lgn60h00	L39	143	5	i.v. equi	0	3.75	y	ON	36	n	no
123	lgn60h01	L39	143	4	i.v. equi	1.5	3.75	y	ON	37	n	no
124	lgn60h02	L39	143	4	i.v. equi	0	3.75	y	ON	36	n	no
125	lgn60h03	L39	143	5	i.v. equi	1.5	3.75	y	ON	36	n	no
126												
127	lgn61h00	L40	144	3	i.v. equi	1.5	3.67	y	OFF	30	y	yes
128	lgn61h01	L40	144	3	i.v. equi	0	3.67	y	OFF	40	y	yes
129	lgn61h02	L40	144	3	i.v. equi	1.5	3.67	y	OFF	35	y	yes
130												
131	lgn62h00	L41	144	5	i.v. equi	1.5	3.8	y	ON	38	y	yes
132	lgn62h01	L41	144	5	i.v. equi	0	3.8	y	ON	38	y	yes
133	lgn62h02	L41	144	4	i.v. equi	1.5	3.8	little	ON	38	y	yes
134	lgn62h03	L41	144	4	i.v. equi	10	3.8	little	ON	38	y	yes
135	lgn62h04	L41	144	1	i.v. equi	90	3.8	little	ON	NA	n	yes
136	lgn62h05	L41	144	5	i.v. equi	0	3.8	y	ON	38	y	yes
137	lgn62h06	L41	144	5	i.v. equi	1.5	3.8	y	ON	38	y	yes
138												
139	lgn63h00	L42	144	2	i.v. equi	0	3.86	little	OFF	45	y	yes
140	lgn63h01	L42	144	2	i.v. equi	1.5	3.86	little	OFF	50	y	yes
141	lgn63h02	L42	144	2	i.v. equi	10	3.86	little	OFF	NA	y	yes
142	lgn63h03	L42	144	2	i.v. equi	1.5	3.86	little	OFF	40	y	yes
143	lgn63h04	L42	144	2	i.v. equi	0	3.86	little	OFF	50	y	yes
144	lgn63h05	L42	144	2	i.v. equi		3.86	little	OFF	NA	y	yes
145												
146	lgn64h00	L43	144	4	i.v. equi	0	4.56	y	OFF	40	n	yes
147	lgn64h01	L43	144	4	i.v. equi	1.5	4.56	y	OFF	35	n	yes
148	lgn64h02	L43	144	4	i.v. equi	1.5	4.56	y	OFF	20	n	yes
149	lgn64h03	L43	144	2	i.v. equi	90	4.56	y	OFF	NA	n	yes
150												

	A	B	C	D	E	F	G	H	I	J	K	L
151	lgn65h00	L44	144	5	i.v. equi	0	4.64	y	OFF	40	n	yes
152	lgn65h01	L44	144	4	i.v. equi	1.5	4.64	y	OFF	38	n	yes
153	lgn65h02	L44	144	3	i.v. equi	1.5	4.64	y	OFF	38	n	yes
154	lgn65h03	L44	144	2	i.v. equi	10	4.64	little	OFF	NA	n	yes
155	lgn64h04	L44	144	1	i.v. equi	90	4.64	y	OFF	NA	n	yes
156	lgn65h05	L44	144	5	i.v. equi	0	4.64	y	OFF	40	n	yes
157												
158	lgn66h00	L45	144	5	i.v. equi	0	4.42	y	ON	30	n	yes
159	lgn66h01	L45	144	5	i.v. equi	1.5	4.42	y	ON	30	n	yes
160	lgn66h02	L45	144	1	i.v. equi	10	4.42	little	ON	NA	y	yes
161	lgn66h03	L45	144	1	i.v. equi	90	4.42	little	ON	NA	n	yes
162	lgn66h04	L45	144	4	i.v. equi	0	4.42	y	ON	30	n	yes
163	lgn66h10	L45	144	4	i.v. equi	0	4.42	y	ON	30	n	yes
164												
165	lgn67h01	L46	144	2	i.v. equi	1.5	4.22	y	OFF	25	y	yes
166	lgn67h02	L46	144	2	i.v. equi	10	4.22	y	OFF	NA	n	yes
167	lgn67h03	L46	144	2	i.v. equi	0	4.22	y	OFF	30	n	yes
168	lgn67h04	L46	144	2	i.v. equi	1.5	4.22	y	OFF	25	n	yes

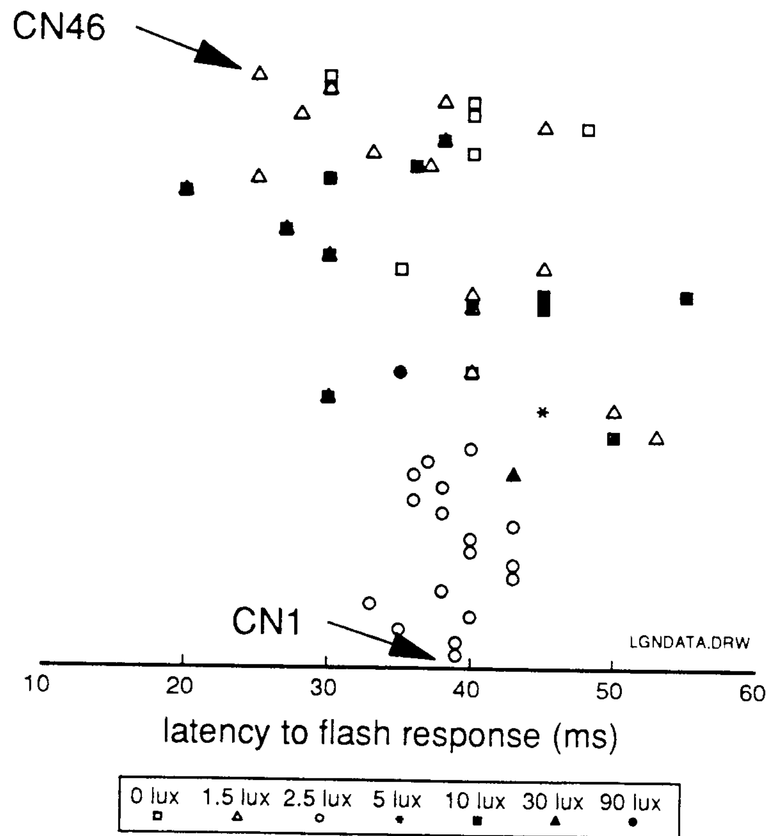


Fig. 6.0. Scatter plot shows the latencies of the ON- and OFF- like LGN neuronal responses to flash stimulation. For ON cells, latency represents the time to the excitatory peak. For OFF cells, latency is time to the inhibition. See Table 6.2 for numerical data.

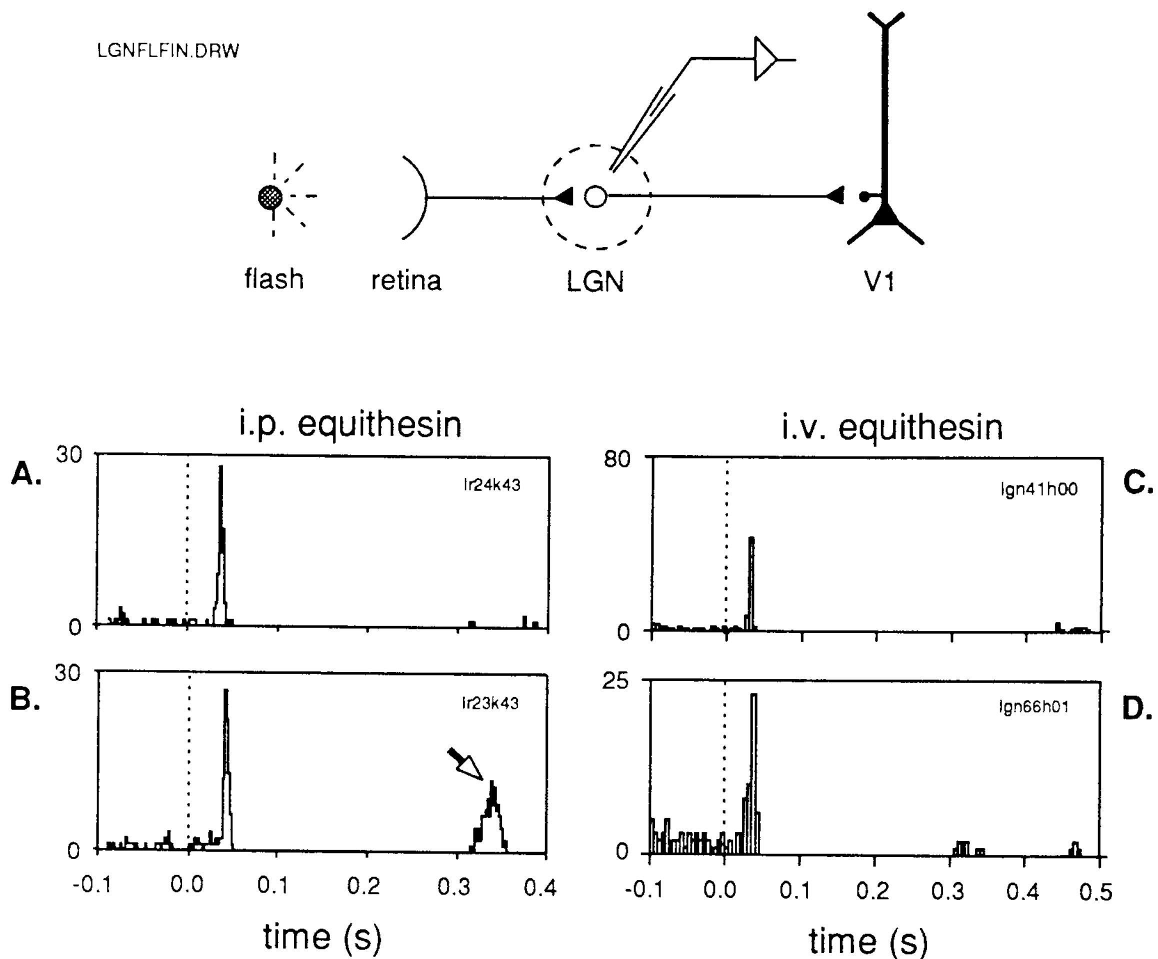


Fig. 6.1. Typical extracellular responses of 4 ON-like dLGN neurones to strobe-flash stimulation of the contralateral eye (**A-D**). Schematic (shown above the 4 PSTH examples) represents the experimental protocol with the recording electrode positioned in the LGN. Response of cells **A** & **B** were recorded from animals anaesthetised with equithesin administered intraperitoneally. Response of cells **C** & **D** were recorded from animals receiving continuous intravenous administration of equithesin. Irrespective of the route of equithesin administration, flash stimulation produced a brief excitatory phase (8-16 ms duration) after a latency of 30-40 ms. This was always followed by a sustained inhibitory phase lasting 250-400 ms. Response of cell **B** shows a prominent late excitatory discharge (LD) (arrowed) that interrupted the sustained inhibition. Flash stimuli applied at time zero (as indicated by the dashed line). Background illumination kept at 1-3 lux.

The sensitivity of the flash-evoked LGN response to the level of background (or surround) illumination was tested in 11 of the 30 ON-like and 10 of the 11 OFF-like neurones (see Table 6.2 for complete data set). Like the cortical recordings (chapter 5), these were obtained only from rats receiving continuous intravenous administration of equithesin. In this section, we present (in PSTH format) 4 ON-like and 2 OFF-like neurones which are fully representative of the entire sample.

The 4 ON-like examples are shown in Fig. 6.2 (A-D), Fig. 6.3 (A-E), Fig. 6.4 (A-C), and Fig. 6.5 (A-C). Like those examples presented in Fig. 6.1 above, ON-like cells showed an early excitatory discharge (ED), 25-35 ms from application of the strobe-flash stimuli. No sustained re-excitatory phase was observed in ON-like dLGN neurones with flash stimulation, irrespective of the level of background illumination (BI) (see Fig. 6.2 - 6.5). Like the cortical neurones (chapter 5), these thalamic neurones responded best at subdued levels of background illumination i.e. 0 - 5 lux. The temporal form of the response in cortical and these LGN neurones were completely different. In ON-like LGN neurones, provided that the background illumination was optimal, an ED always emerged with flash stimulation (e.g. see Fig. 6.2 A & B; Fig. 6.3 A, B, C & E). In such instances, the ED was followed by a sustained inhibition, 250-400 ms in duration. At sub-optimal levels of background illumination (typically 10 - 90 lux), an ED failed to emerge upon flash stimulation (e.g. see Fig. 6.2 C & D; Fig. 6.3 D). Late excitatory discharges (LDs) emerged 200-350 ms from the ED (e.g. see Fig. 6.3). In just one LGN neurone, did we find the LD having a magnitude larger than the ED (Fig. 6.4). The LD also occurred much earlier than that seen in other recorded LGN neurones. The latency to the LD of this neurone also appeared to be moderately sensitive to the level of background illumination. Even in this *most extreme example* (Fig. 6.4), the latency to the LD shifts by a mere 20-25 ms from one level of surround illumination to another. Even then, no sustained re-excitatory events emerged with flash stimulation. The pronounced LD simply represented a brief interruption of the sustained post ED phase of inhibition.

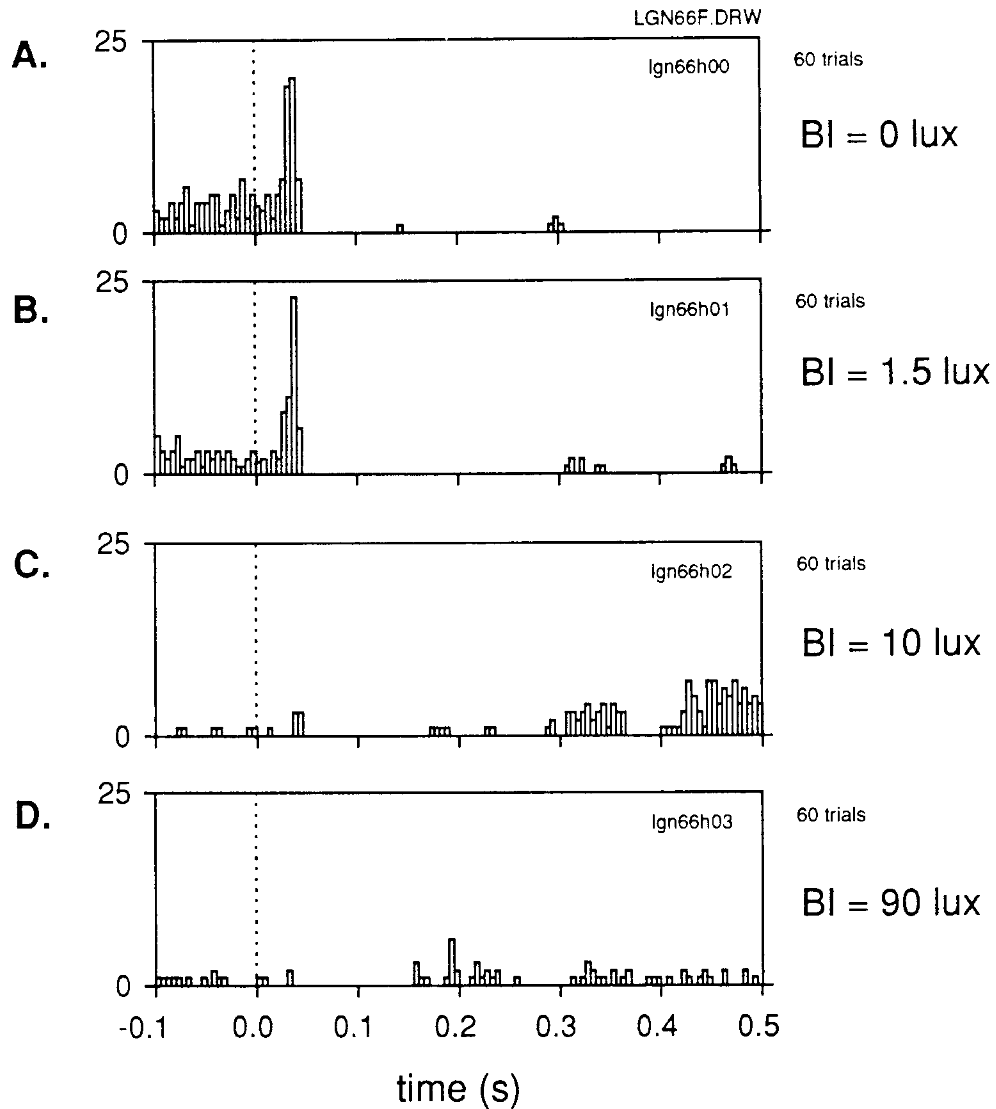


Fig. 6.2. Typical extracellular responses of a single ON-like LGN neurone to flash stimulation of the contralateral eye (**A**-**D**). Background illumination (BI) levels were tightly controlled and systematically altered from 0 through to 90 lux. This and subsequent examples (Fig. 6.2 - 6.4) obtained from animals anaesthetised with equithesin (i.v). Flash stimuli in all cases applied at time zero (as indicated by the dashed line). Each PSTH represented the cumulative response over 60 trials. **A**. Flash evoked response with BI at 0 lux was characterised by an early discharge (ED) after a latency of 30 ms. Thereafter, a sustained period of quiescence (450 ms) was observed. **B**. With BI at 1.5 lux, the flash-evoked ED and the sustained inhibition was maintained. Note however, that the spontaneous discharge activity (as reflected in the control period) decreased when shifting from 0 to 1.5 lux. **C**. BI of 10 lux renders this cell largely unresponsive to the flash stimuli i.e. ED disappeared. However, some late rebound activity emerged. Note that the spontaneous discharge in the control period decreased even further at this level of background illumination. **D**. BI of 90 lux produced a weak response with no distinct phases of excitation and inhibition. Irrespective of the level of background illumination, secondary or sustained excitatory events remained absent in this LGN neurone with flash stimulation.

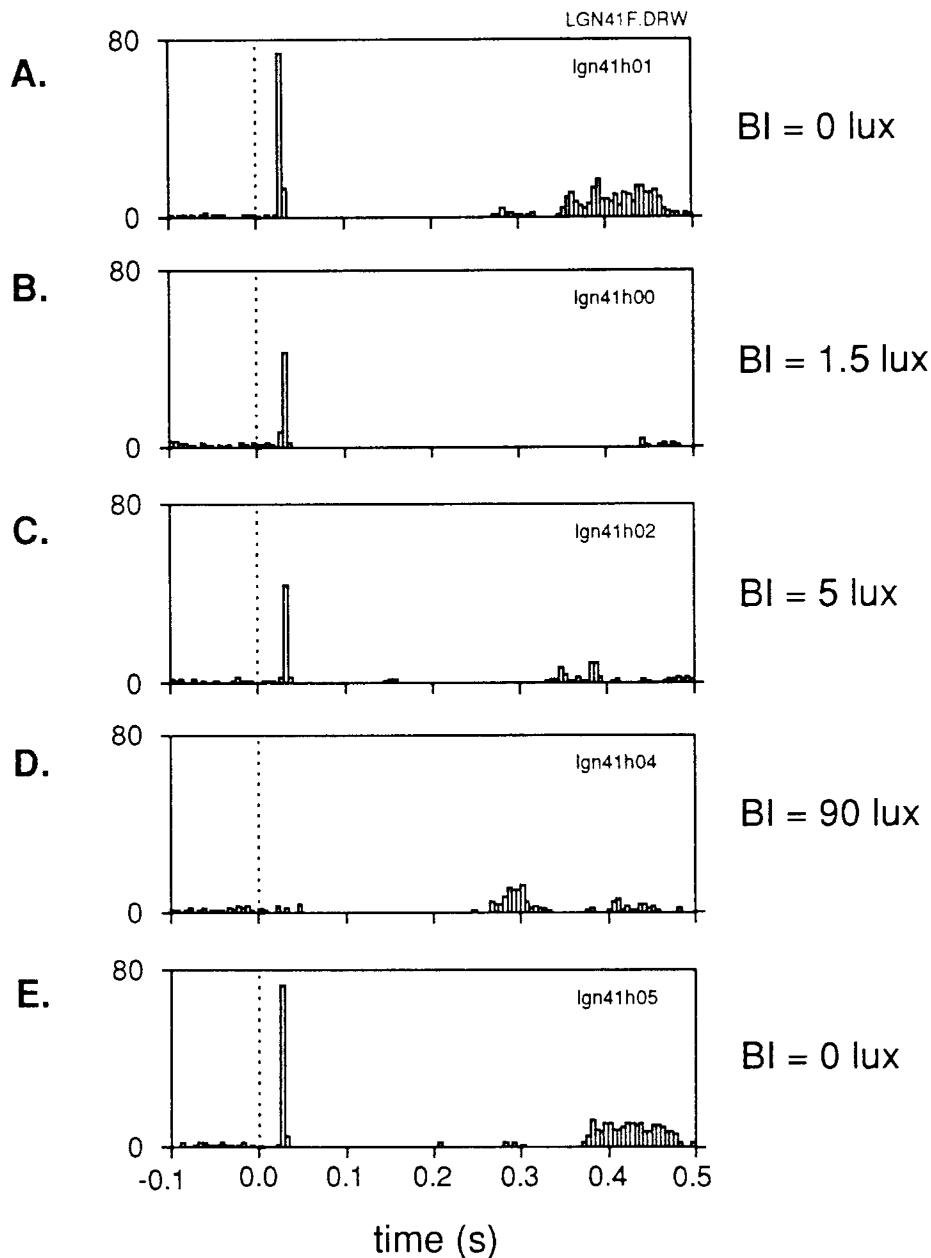


Fig. 6.3. Extracellular response of another ON-like LGN neurone to flash stimulation with different levels of background (or surround) illumination. **A**. Flash evoked response with background illumination (BI) at 0 lux. As observed in the previous example, application of the flash stimulus evoked a prominent ED (here, 25 ms latency) followed by a sustained inhibition (250-300 ms). A late excitatory discharge (LD) completed the response. **B**. ED maintained with BI = 1.5 lux, albeit its magnitude slightly attenuated. Post ED inhibition was extended to 380-400 ms with little LD activity. **C**. ED maintained with BI = 5 lux. **D**. ED disappeared with BI = 90 lux. **E**. Return to BI of 0 lux, once again produced a prominent early discharge with a sustained period of quiescence. Clearly, no flash-evoked secondary excitatory events emerged in this LGN neurone. Rather a sustained inhibition was the norm, especially at background illuminations from 0 - 5 lux.

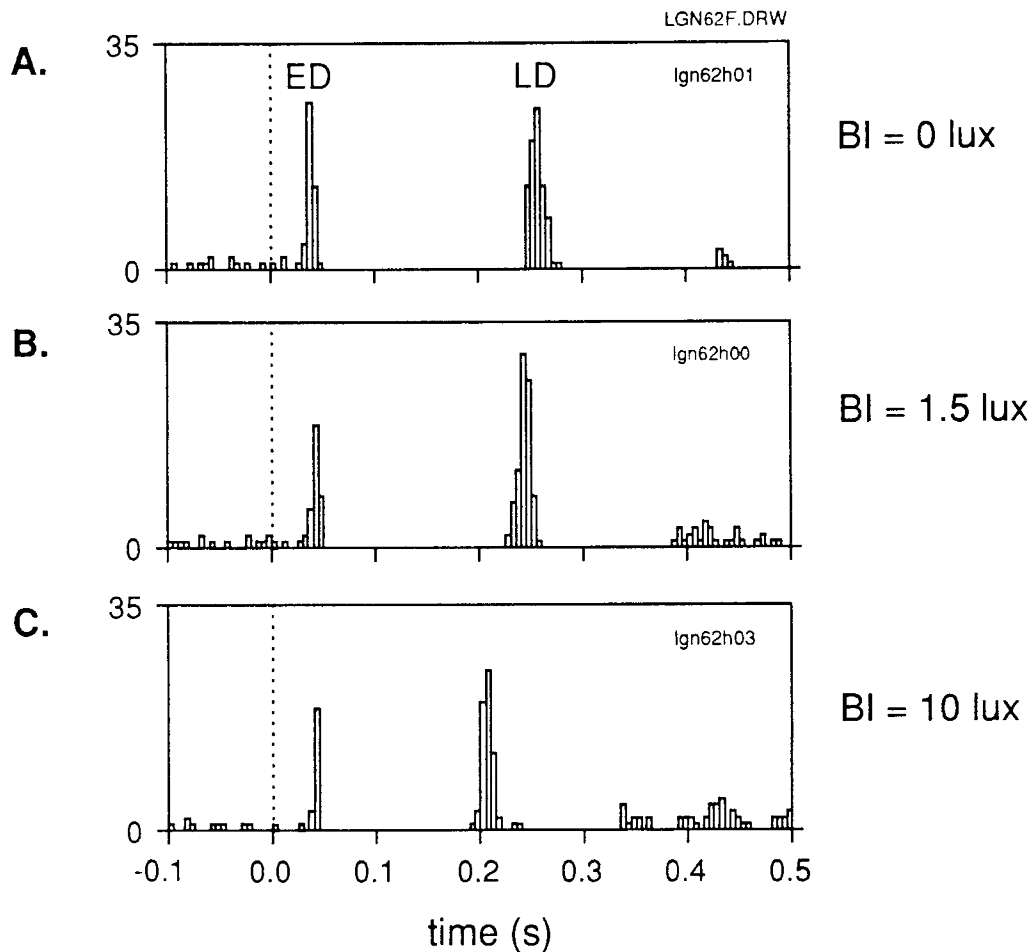


Fig. 6.4. Flash-evoked response of a single ON-like LGN neurone (**A**-**C**). This was the only LGN neurone where the LD was greater in magnitude than the ED. The LD also interrupted the post ED inhibition much earlier than that seen in other ON-like LGN cells. The latency to the ED (35 ms) was unaffected by changing the level of background illumination (BI). The latency to the LD was somewhat influenced by the level of BI. With BI = 0 lux, the flash evoked LD emerged within 200 ms of the post ED inhibition (**A**). With BI = 1.5 lux (see **B**), the flash evoked LD occurred earlier than in **A**, 176 ms into the post ED inhibitory phase. With BI = 10 lux (see **C**), the flash evoked LD emerged even earlier, 154 ms into the post ED inhibition. So overall, the latency to the LD decreased in 20-25 ms intervals from 0 to 1.5 to 10 lux. The neurone was completely unresponsive to flash stimuli with BI = 90 lux (not shown).

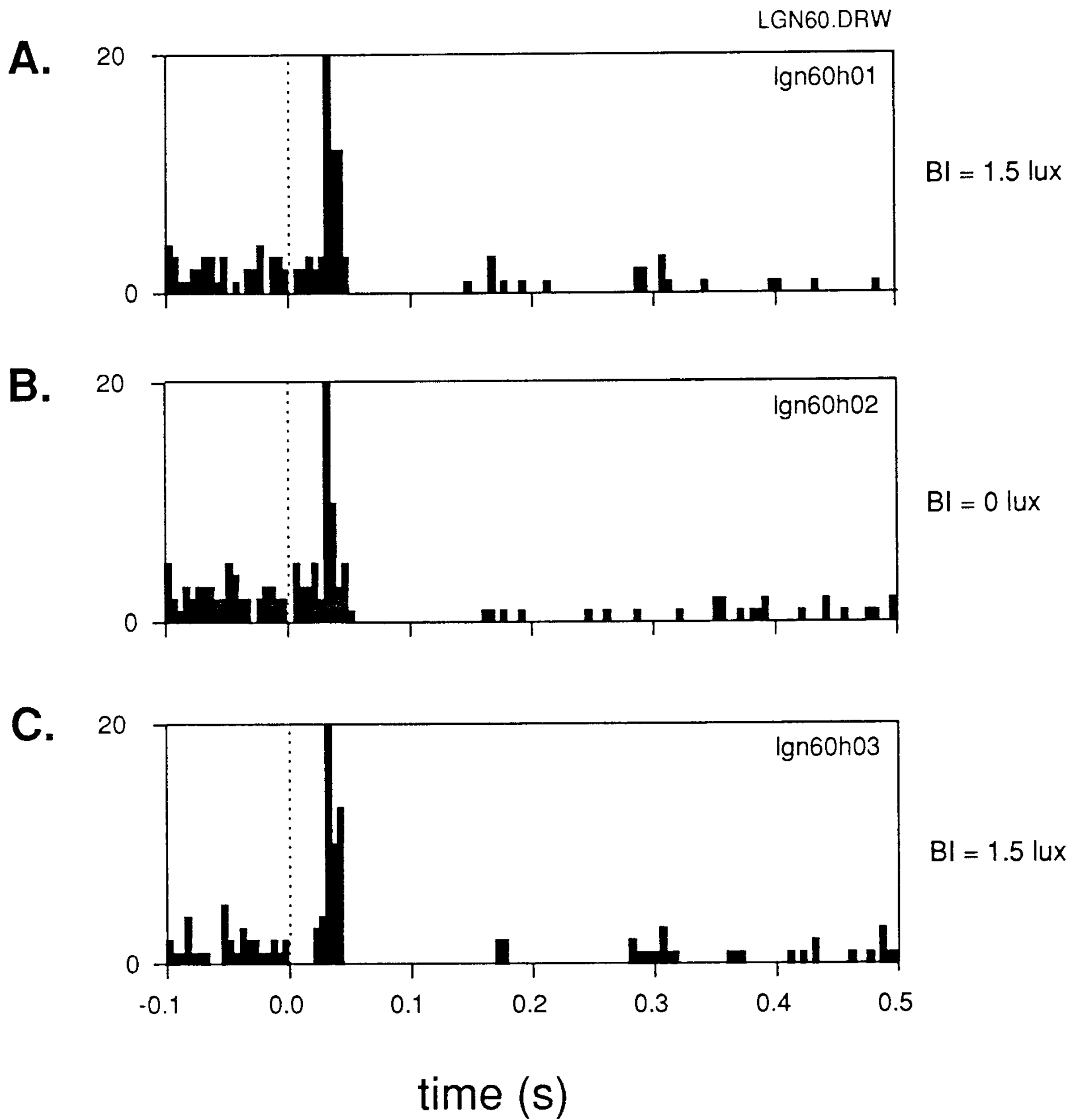


Fig. 6.5. Typical extracellular responses of another ON-like LGN neurone to flash stimulation. Unlike the previous examples, the responsiveness of this cell was not sensitive to changes in the level of background illumination. However, consistent with the population of recorded geniculate neurones, a sustained period of quiescence followed the early excitatory peak.

In all of the ON-like neurones tested in this study, there was no marked change in latency to the ED in the face of changing background illumination.

All 10 of the tested OFF-like neurones switched off (became inhibited) after a latency of 25-35 ms from the application of the flash stimuli, with background illuminations of 0 - 10 lux. This flash-evoked inhibition was maintained for 200-400 ms. The responses of two OFF-like LGN neurones (in PSTH format) are shown in Fig. 6.6 (A-E) and Fig. 6.7 (A-C).

The spontaneous discharge of the first OFF-like unit (Fig 6.6) was greatest at 0 lux, as reflected in the control period (see A & E). Consequently, the OFF-like flash evoked response was most dramatic at 0 lux. This neurone (like the remainder of the sample of 10) failed to show a prominent flash evoked OFF-like response with background illumination at 90 lux.

Like many of the ON-like cells (e.g. Fig. 6.3), the flash response of these OFF-like cells (Fig. 6.6 & 6.7) were recorded at different levels of background illumination, e.g. from 0 through 90 lux, and then re-recorded at 0 lux. This was to ensure that any flash-evoked sensitivity of the temporal pattern of the responses with different levels of background illumination (BI) was a function of the level of BI, and not as a result of fluctuating levels of anaesthesia or injury of the tested units. As observed in the ON-like neurones presented above, no sustained re-excitatory events emerged in these OFF-like thalamic neurones, irrespective of the level of background illumination.

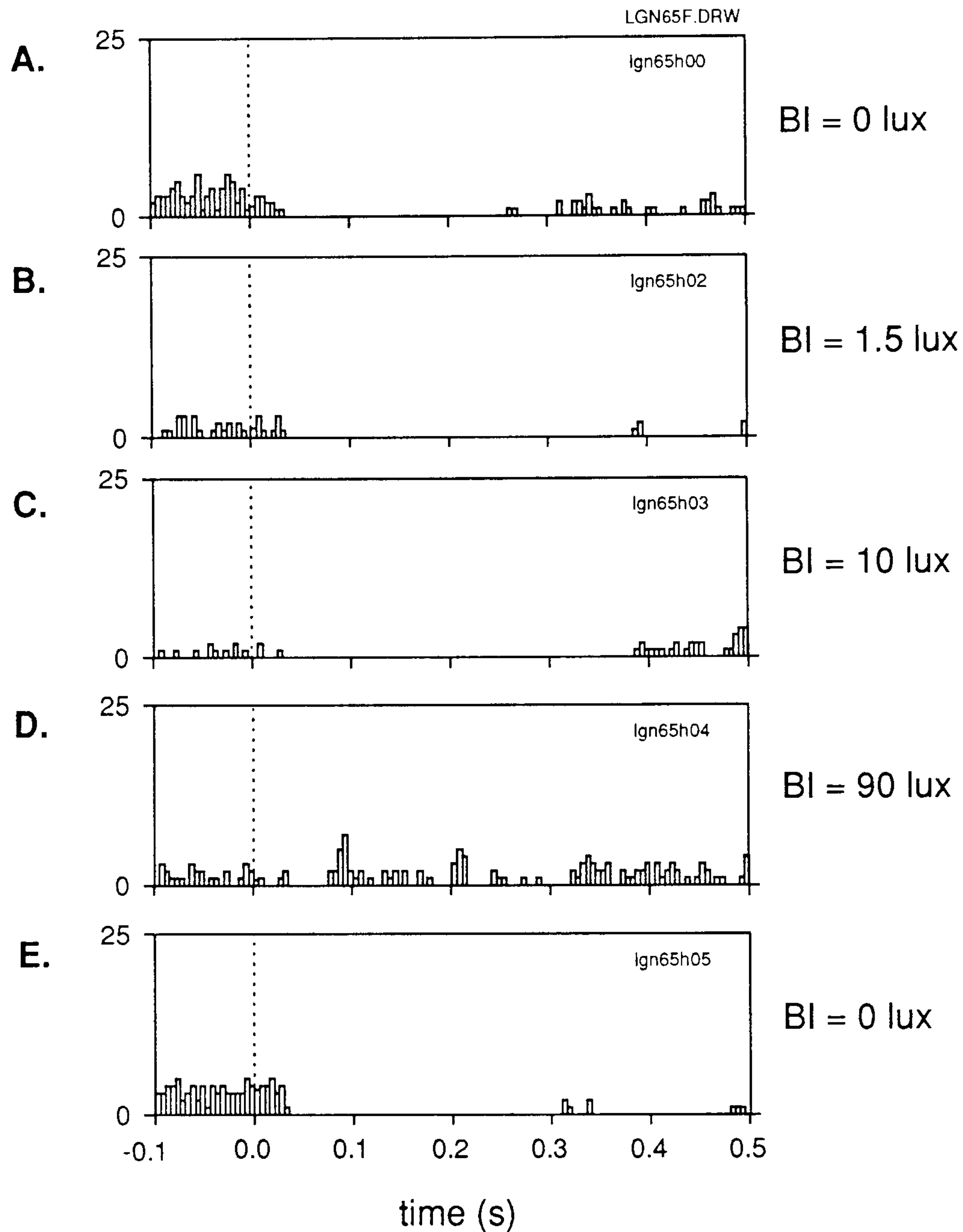


Fig. 6.6. Typical extracellular responses of a single OFF-like LGN neurone to flash stimulation of the contralateral eye (**A**-**E**). The OFF-like neurone switched off after a latency of 35 ms from the flash stimulus. This appeared to be consistent from 0 through to 10 lux of background illumination (BI) (**A**-**C**). The inhibition was maintained for 250-400 ms. Background illumination of 90 lux did not produce any response (**D**), as the level of activity in the test period was similar to that in the control. Return to BI of 0 lux (**E**), once again, produced the most optimal (OFF-like) response. Like the ON-like cell shown in Fig. 6.2, the level of spontaneous discharge (as reflected in the control period) was dependent on the level of BI, and was highest at 0 lux (complete darkness).

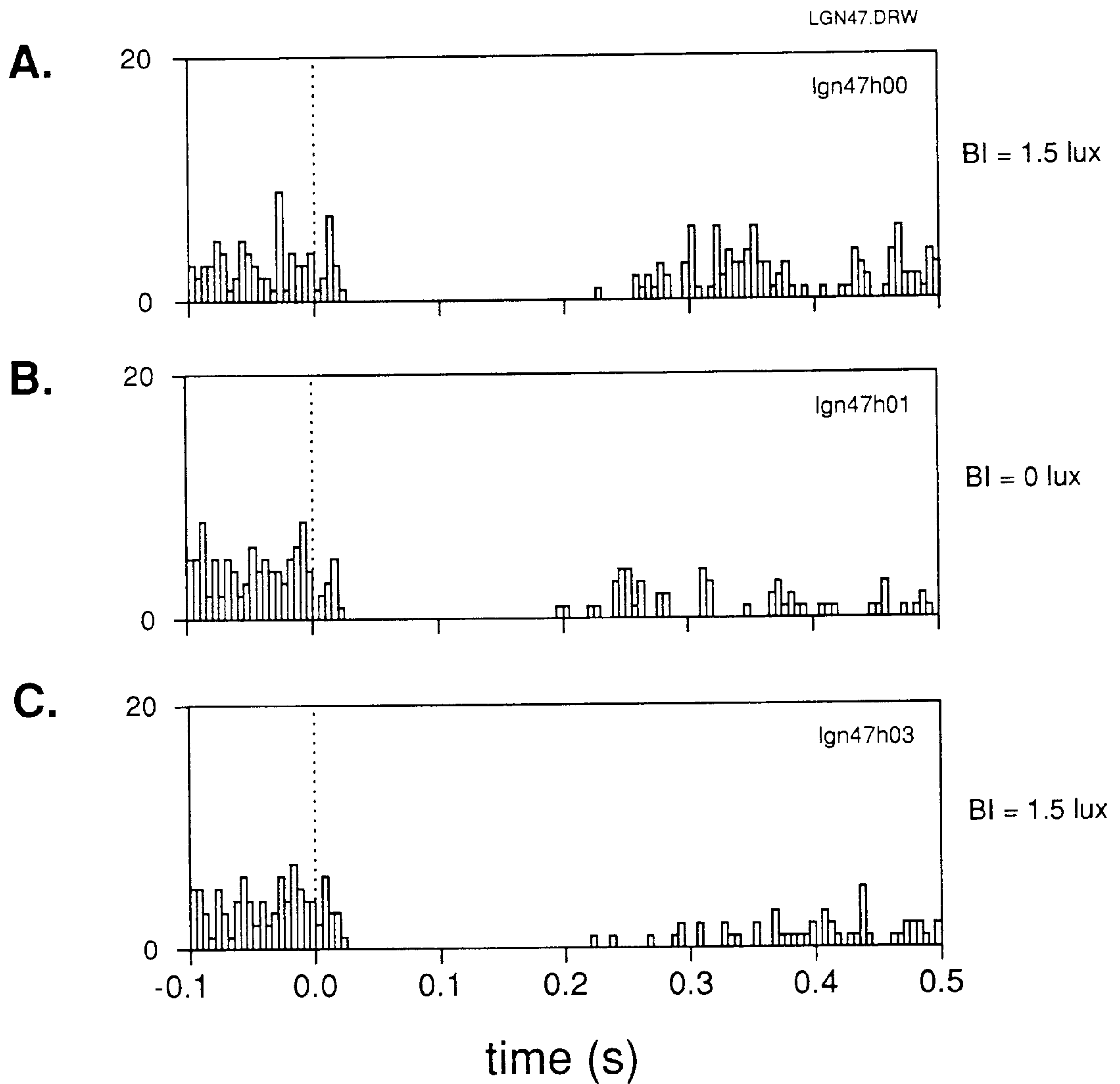


Fig. 6.7. Another typical response of a single OFF-like LGN neurone to flash stimulation (**A-C**). The OFF-like neurone switched off after a latency of 25 ms from the flash stimulus. The inhibition was maintained for 180-220 ms.

6.5. Discussion

6.5.1. *General*

The results show that dLGN neurones can be classified as either ON or OFF types, based on their response to visual spot or strobe-flash stimulation.

The response of ON-like dLGN neurones to strobe-flash stimulation of the contralateral eye was characterised by a primary excitatory or early discharge (ED) phase after a latency of 25-40 ms from the onset of the stimulus. Thereafter, a 200-400 ms period of inhibition was observed. In 70% of the sample, a rebound excitatory or late discharge (LD) phase completed the response. OFF-like dLGN neurones simply switched off (were inhibited) after a latency of 25-35 ms from the application of the strobe-flash stimuli. This flash-evoked inhibition was maintained for 200-400 ms.

It is clear that sustained secondary excitatory activity, as observed in striate cortical neurones (chapter 5), are completely absent in ON and OFF like dLGN neurones, irrespective of the level of background illumination.

6.5.2. *Comparison of the present data with earlier studies*

The most extensive literature on single flash responses of rat geniculate neurones comes from the work of Fukuda *et al* (1973, 1975). Their findings have been reviewed in chapter 2. Fukuda *et al* (1973, 1975) recorded the flash-evoked responses from the lateral geniculate body (LGB) which is equivalent to the lateral geniculate nucleus (LGN), the latter term being preferred in the current literature. In principle, their findings are consistent with the observations in this study, at least in terms of the form of the temporal response to flash stimulation, for the ON-like LGN neurones (e.g. compare Fig. 6.1 with Fig. 2.4). Fukuda *et al* (1973, 1975) did not report the presence of any OFF-like LGN neurones in their urethane anaesthetised rats. No reasons for the absence of OFF-like LGN neurones were given.

Like the findings of Fukuda *et al* (1975), the present study showed that it was rare to observe more than a single late excitatory discharge (LD) phase in the flash evoked LGN response, irrespective of the level of background illumination. In fact, for the first 500 ms of the test period, we report no second LD in any of the 55 LGN neurones tested in this study. Our data show

that a single flash-evoked LD was observed in 70% of tested LGN neurones. Fukuda *et al* (1973, 1977) did not report the percentage of geniculate neurones that respond with LDs, but they give the impression that all tested neurones displayed at least a single LD. The significance of this brief LD is not known, but its origin might be from the cortex itself because there are extensive feedback connections from the cortex, back to the thalamic nuclei (see chapter 2). It could also be argued that the LD emerges intrinsically upon the removal of inhibition, because the threshold for action potential generation is sometimes reduced with a sustained hyperpolarising inhibition. An emergence of a rebound excitation following sustained inhibition is commonly referred to as the "anodal break phenomenon" (see Ranck, 1981).

In this study, we recorded the flash responses from only dLGN neurones. The LGN has two divisions, a dorsal (dLGN) and a ventral (vLGN) one (see chapter 2). We chose to record from only the dLGN, because it is only neurones from this thalamic nucleus that project to the visual cortex (see chapter 2). While the vLGN receives retinal input, their neuronal output is purely sub-cortical, to other thalamic nuclei (Sefton & Dreher, 1985; Sherman & Koch, 1990).

Fukuda *et al* (1975) attempted to classify the flash-evoked response of LGN relay cells into fast and slow types. This classification is inappropriate because there was a large overlap of the response characteristics between these two sub-populations (see chapter 2, section 2.3.2). For example, the mean latency to the ED of the fast type cells was 38.0 ± 10.3 ms whereas the mean latency to the ED of the slow type cells was 44.7 ± 12.2 ms. This represents a mere 6.7 ms difference between the two groups, and it is most likely that this difference is statistically insignificant because the standard deviations of the means are clearly larger than the difference of the means. We did not attempt to classify the ON-like LGN units recorded in the present study into fast and slow types. This is because there was a small range in the latency to the ED of the flash-evoked responses in our sample i.e. 25-40 ms. Furthermore, our intention in recording from single LGN units was not to produce a detailed classification of the various classes of LGN neurones. As outlined in chapter 1 and the introduction to this chapter, the objective of recording from the dLGN in this study was merely to test for the possible existence of protracted excitatory events, similar to that observed in visual cortical neurones (chapter 5).

This study, and that of Fukuda *et al* (1975), demonstrates that sustained excitatory events do not exist in the LGN, irrespective of the level of background illumination. Instead, LGN neurones are inhibited during much of the time that the secondary phase of excitation in cortical neurones is evolving and being propagated. Together, these findings suggest that instead of the thalamic input, the rich intra-cortical circuitry itself might be responsible for generating the cortical re-excitatory response.

Chapter 7

GENERAL DISCUSSION

This chapter discusses aspects of cortical processing that are related to both the cortical (chapter 5) and thalamic (chapter 6) data. Some attention is paid to the design of the present study with reference to the stimulus series. Of particular importance will be the theoretical significance of this work in terms of past and current views on cortical processing. The chapter will provide possible explanations for the difference in the flash versus electrically evoked cortical responses. Proposed models and ideas presented in this chapter arise from a combination of a rich source of established microanatomical data from previous studies (chapters 2 & 3) and the electrophysiological data of this study (chapters 5 & 6). Based on the findings of the present study, the chapter closes by outlining the direction for future work.

7.1. Advantages of using transient as opposed to conventional sustained stimuli in analysing cortical processing

Previous studies always attempted to record the most optimal response to the most optimal visual stimulus for a particular neurone e.g. circular spot stimuli for thalamic neurones and variants of elongated bar-like stimuli for cortical neurones. These in turn would be presented at the most optimal velocity and directionality. To demonstrate this selectivity, these stimuli would necessarily have to be *applied* for at least a few seconds per trial i.e. a few thousand milliseconds. Similarly, the neuronal discharge of these neurones to these stimuli would have to be *recorded* for a few seconds per trial. Consequently, any inhibitory phases of tens of milliseconds would be completely masked in the test period of a few seconds. Furthermore, the performance of cortical neurones would not necessarily reflect intracortical processing, as the sustained stimuli would continuously influence sub-cortical (i.e. retinal and thalamic) circuits. A point of particular importance that is absent in all the literature is that of artefacts of adaptive inhibition that arise sub-cortically. Virtually all single-unit electrophysiological studies of the visual system are performed in paralysed, anaesthetised animals. Paralysis is required to fully immobilise eye oscillations or saccades that might distort the accurate mapping of the receptive field structure of the recorded unit. It is

also critical that alterations in the position of the eye and therefore of the receptive fields of individual units be kept to an absolute minimum in any form of multiple trial analyses, as that performed in this study (see chapter 4). Anaesthesia alone does not ensure the removal of saccades. In the awake, unanaesthetised state, micro-saccades prevent adaptation or adaptive inhibition at the retinal level. Practically, if these saccades were to be removed in the awake, unparalysed state, it would ensure that the perception of stationary stimuli by the subject would disappear completely, within a second, until either the stimulus position or the subject's head is altered (see Mahowald & Mead, 1991). The use of conventional natural stimuli that are applied for a few seconds ensures that retinal adaptive inhibition is activated in such paralysed and anaesthetised states. On the other hand, the use of transient natural stimuli (e.g. strobe-flashes) in similar states, as done in the present study, will prevent retinal adaptive inhibitory mechanisms from being engaged, and so allow for the visibility of true intracortical excitatory and inhibitory events.

The use of transient *electrical* stimuli have also proved to be valuable in analysing cortical processing. Even though electrical stimulation is artificial (Ranck, 1981; Douglas & Martin, 1991), it is easily controlled and highly reproducible, and application of such stimuli to the thalamic afferents by-passes any influence from the retinal circuits. So, the approach in the present study was to *trigger* the cortical circuits with transient artificial or natural stimuli and observe the evolution of the response of individual cortical neurones.

The approach in the present study is thus a novel one as it is the first to record the response of cortical neurones to transient electrical and strobe-flash stimulation. It is also the first study to compare the cortical and thalamic response to the same type of transient strobe-flash stimuli. A few earlier studies (Creutzfeldt *et al*, 1969; Kunt & Creutzfeldt, 1971) did record the cortical response to transient natural stimuli, but these were done in isolation i.e. without the response of these neurones to transient electrical stimuli. Other studies (Douglas *et al*, 1989; Douglas & Martin, 1991) have recorded the cortical response to transient electrical stimuli in isolation i.e. without the response to natural transient stimuli. All of these previous studies were performed in cats, and have been reviewed in chapter 3. A single group (Fukuda *et al* 1973, 1975) have recorded the response of thalamic neurones (in rats) to transient flash stimulation, but once again, in isolation i.e. cortical responses to such transient stimuli were not recorded. These

findings were reviewed in chapter 2. The similarities of the findings of these previous studies to the data obtained in this study have already been considered in the discussions of the relevant cortical (chapter 5) and thalamic (chapter 6) data.

7.2. Significance of the difference in the cortical and thalamic response to transient stimuli

The electrophysiological data presented in this study show that cortical processing of transient (< 1 ms) flash or electrical stimuli continue for 160-400 ms. The flash-evoked response of cortical neurones is characterised by secondary excitatory events that continue for 200-300 ms from the application of the transient stimulus. The absence of such sustained excitatory events in LGN relay neurones upon flash stimulation (see Fig. 7.1), confirm that they must originate from intracortical sources. Even though the cortical phase of re-excitation is to a certain extent, modulated by the level of background illumination (chapter 5), the point is that such a sustained re-excitation *never* emerges in LGN neurones, irrespective of the level of background illumination. These electrophysiological data provide ample evidence that the rich intracortical circuitry itself, which show massive positive feedback connections between spiny pyramidal (i.e. excitatory) neurones throughout the cortex, is actively involved in the processing of natural stimuli. In support of the electrophysiological data of the present study, the thalamic circuits (e.g. see Fig. 2.5 & 2.6) simply do not have the machinery (or physical structure) to generate massive re-excitation. Thalamic neurones do not receive a rich source of excitatory synaptic contacts from other neighbouring thalamic neurones of the same nucleus e.g. dLGN. Rather, they are interconnected by an elaborate system of inhibitory synapses from the dendrites of adjacent and neighbouring thalamic neurones; and from the cortex itself, albeit indirect via the RNT (see chapter 2).

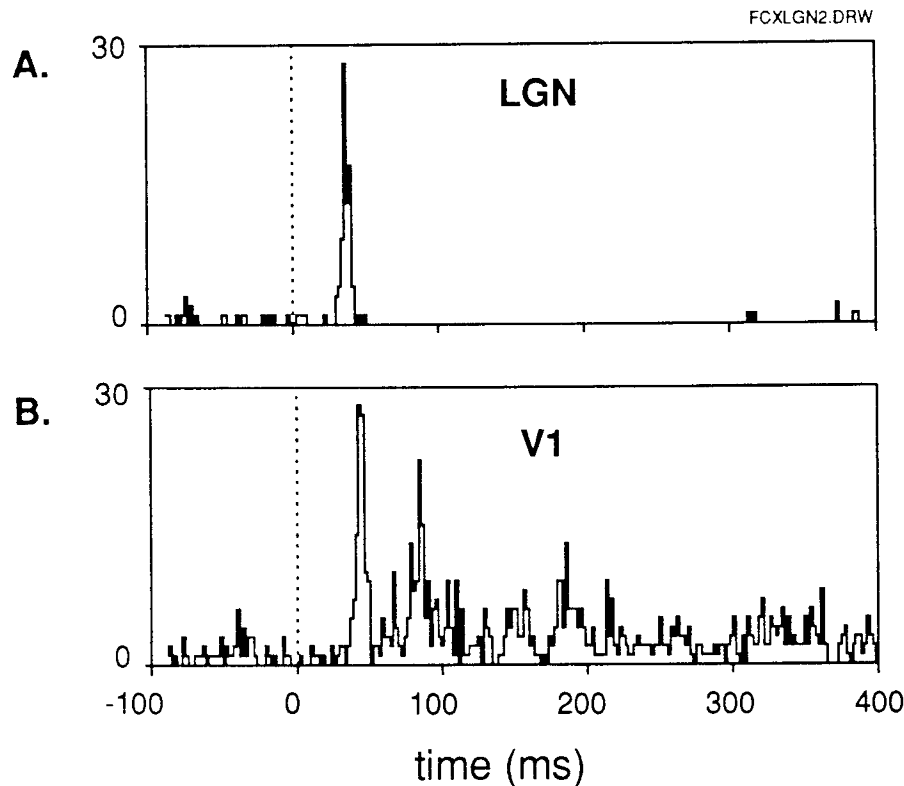


Fig. 7.1. Typical extracellular response of a LGN (**A**) and a visual cortical neurone (**B**) to strobe-flash stimulation. Stimulus indicated by dashed line at time zero. Sustained re-excitatory events, as that observed in visual cortical neurones, are completely absent in LGN neurones.

The above electrophysiological data go against classic models e.g. mechanism of orientation selectivity (see Fig. 3.4) that over-emphasize the role of the thalamus in shaping the receptive field structure and response of cortical neurones to such selective stimuli. Another implication of the flash-evoked sustained re-excitatory component is that it opposes the classic view that cortical processing relies on precise and rapid timing between individual synaptic inputs, which are in the order of tens of milliseconds, not hundreds of milliseconds. So, the precise timing required for many perceptual tasks of the cortex is expected to arise as a property of intracortical microcircuit operations, which evolve over a few hundred milliseconds. It could be argued that the intracortical phase of re-excitation arises as a result of adjacent cortical microcircuits or modules, and possibly other visual areas e.g. V2 and V3 operating simultaneously. Engel *et al* (1990) have shown, that in lightly anaesthetised cats, individual neurones in different visual cortical areas often

synchronise their firing to the same stimulus presented. The degree to which part of the striate (V1) re-excitatory component may arise from extrastriate (e.g. V2 & V3) cortical areas in the rat visual system can be investigated in future studies. The important point of the present study is that the phase of secondary re-excitation is nevertheless still *intracortical*, not thalamic. Today, such a mass-action idea is favoured over a strict serial and hierarchical type of processing e.g. from V1 to V2 to V3, as proposed by Hubel & Wiesel (1962). Also see Hubel (1988) for a review. Indirect support from computer simulation studies show that using serial processing with hierarchical wirings (like that specified by Hubel & Wiesel) result in signal transmission and integration time that is just too long (Churchland & Churchland, 1990). Rather, models that rely heavily on parallel and cross-connectivity e.g. 3 layer neural networks (Lehky and Sejnowski, 1988) have proved to be more fruitful, not only in their dramatic improvement in speed but also that the speed of processing is entirely independent of both the number of units involved in each layer and the complexity of the function they are computing.

The above-mentioned hypothetical circuits that were supposedly responsible for selective cortical responses like orientation preference arose purely from receptive field mapping studies (see Hubel, 1988; for a review). Yet they remained ingrained in modern texts of neurophysiology (Carpenter, 1990). Understandably, during the 60s and 70s, the actual microcircuitry of the cortex was a complete enigma. Today, we have a good idea of the physical structure of the basic cortical microcircuits (Fig. 3.3). Even though the electrophysiological data presented in this study together with microanatomical data from earlier studies, clearly contradict the classic hierarchical models, further research is required before *exact* circuit mechanisms that are responsible for the generation of orientation selectivity of cortical neurones are proposed. This study deliberately avoided assessing the degree of orientation tuning of the sampled visual units. The issue of the degree of stimulus selectivity of individual cortical neurones is clearly of little concern to us at this early stage of analysing intracortical microcircuit processing.

While an in depth knowledge of the stimulus selectivity of individual thalamic and cortical neurones has contributed to the understanding of various aspects of visual function (see chapters 2 & 3), it has provided little insight into the nature of microcircuit operations in cortical processing. However, the use of transient stimuli in this study have revealed cortical evoked responses that arise as a property of intracortical circuit operations.

7.3. Significance and possible explanations for the difference in the flash versus electrically evoked cortical response

The results of this study show that there is a clear and consistent difference in the response of cortical neurones to transient natural (strobe-flash) and artificial (electrical) stimulation (chapter 5). Electrical stimulation produced a protracted inhibitory phase (197 ± 61 ms) followed by some rebound (excitatory) activity. Strobe-flash stimulation however, produced primary and secondary excitatory events extending to 200-300 ms, irrespective of the type of anaesthetic used. No protracted level of inhibition was observed either before or after these excitatory events. Electrical stimulation is clearly artificial, after all, it is a novel stimulus to the visual system, and it results in the spatial and temporal synchronous activation of the stimulated fibres (Ranck, 1981; Douglas *et al*, 1989; Douglas & Martin, 1991). Nevertheless, the important implication of the difference in the flash and electrically evoked cortical response is that even though substantial inhibition is available to cortex, only a small fraction of this inhibitory capacity is utilised during natural stimulation.

The profile of the flash stimulus used in this study is similar to that of the electrical stimulus i.e. they are both pulse-like, and both are less than a millisecond in duration. We have shown that the flash evoked sustained re-excitation does not arise from the LGN. We can also be certain that the electrically evoked sustained inhibition is not as a result of a direct inhibitory input from LGN neurones. This is because LGN afferents make only excitatory contacts with their post-synaptic cortical targets (Peters, 1985; Sefton & Dreher, 1985; Dehay *et al*, 1991). This implies that the cortical response to both types of transient stimuli can be explained in terms of the interplay between the excitatory (spiny) and inhibitory (smooth) cell populations in the visual cortex.

With the synaptic organisation and electrophysiological data of the LGN & visual cortex in mind, the following hypothesis is proposed to explain the difference between the flash and electrically evoked cortical responses:

Fig. 7.2 A shows a typical response of a visual cortical neurone to strobe-flash stimulation. Figure 7.2 B shows the proposed model circuit used to explain the flash evoked cortical re-excitatory response. The model has a definite spatial component as reflected by modules 1 and 2. Assume that the recorded unit (as shown in Fig. 7.1 A) is from the stippled spiny cell population of

module 1. Consistent with the microanatomy, LGN afferents make only excitatory contacts with their post-synaptic targets. Spiny cells have the ability to re-excite one another as they are extensively interconnected. A tight coupling between the smooth and spiny cell population exits. Because the spiny (excitatory) cell population is the dominant type in the cortex, accounting for approximately 70-80% of the population, there is always a greater probability that the boutons of the LGN afferents synapse with spiny cells rather than smooth cells. With natural stimulation, it is assumed that only the LGN afferents that synapse on the spiny cell population are engaged. Such a preference is essential if the spiny cells are to display a sustained re-excitatory response. So the LGN triggers the cortical spiny cell population, which evolve a sustained intracortical re-excitatory response because of the massive positive feedback. The smaller smooth (GABAergic inhibitory) cell population is unable to quench the sustained excitation.

Fig. 7.3 A shows a typical response of a visual cortical neurone to electrical pulse stimulation of the LGN. Fig 7.3 B shows the proposed model circuit used to explain the electrically evoked sustained inhibitory response. The same basic intracortical organisation as that used for the circuit in Fig. 7.2 B is used here. The main difference comes from the fact that the only cortical neurones that receive direct synaptic input onto their somata from the LGN afferents, are the smooth (inhibitory) neurones (Freund *et al*, 1985). In addition, the LGN axons that are fed to these smooth cortical neurones are myelinated right up to the boutons. This implies that if the LGN afferents are activated simultaneously, as during electrical stimulation, the smooth cell population would almost always be excited first, before the spiny cell population. This is because the myelinated LGN axons terminating near the axon hillock (of smooth cells) will ensure that the smooth cell activation by-passes the cable properties of the dendrites. This electrically induced excitation of the LGN afferents, ensures that there can be no preferential activation of the spiny cell population (see Fig. 7.3 B). Even though the smooth cells represent just 20-30% of the entire cortical population, they produce a sustained inhibition because they prevent the spiny population from re-exciting themselves. Orthodromic or antidromic spikes preceded the sustained inhibition in 71% of the tested cases in this study (see chapter 5). But this early excitation is always just a single spike. It is more than reasonable to assume that a single spike is allowed to pass through the cortical spiny "gate" before intracortical inhibition sets in. Antidromic activation of layer 6 cortical neurones should not be excluded in contributing to the electrically evoked response. This is because the axons of layer 6

cortical neurones project to the LGN (chapter 2), so electrical stimulation of the LGN will invariably activate some of these axons. The visibility of single electrically evoked antidromic spikes were found in 3 cortical neurones (appendix 2). The main point is that intracortical inhibition sets in early enough to prevent this initial excitation from re-exciting the spiny population of cortical neurones. That the intracortical inhibition sets in early, within a few milliseconds, is clearly visible in the *in vivo* response (e.g. Fig. 7.3 A).

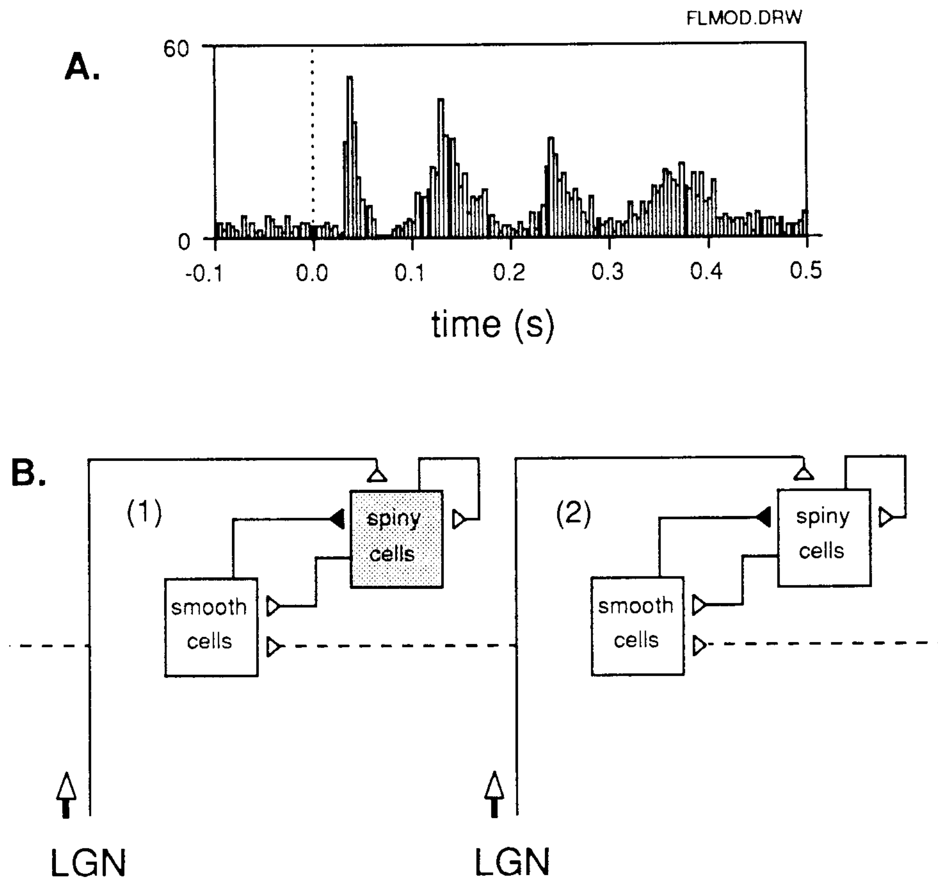


Fig. 7.2. (**A**) Typical extracellular cortical response to strobe-flash stimulation. (**B**) Hypothetical model used to explain the flash evoked *in vivo* re-excitatory response. The model incorporates an interconnected spiny (excitatory) and a smooth (inhibitory) population of neurones. Flash stimulation preferentially activates the thalamic afferents that synapse with the spiny population (solid lines) which has the ability to re-excite itself through positive feedback. The thalamic afferents to the smooth cell population are not activated with natural stimulation, as represented by the hatched lines.

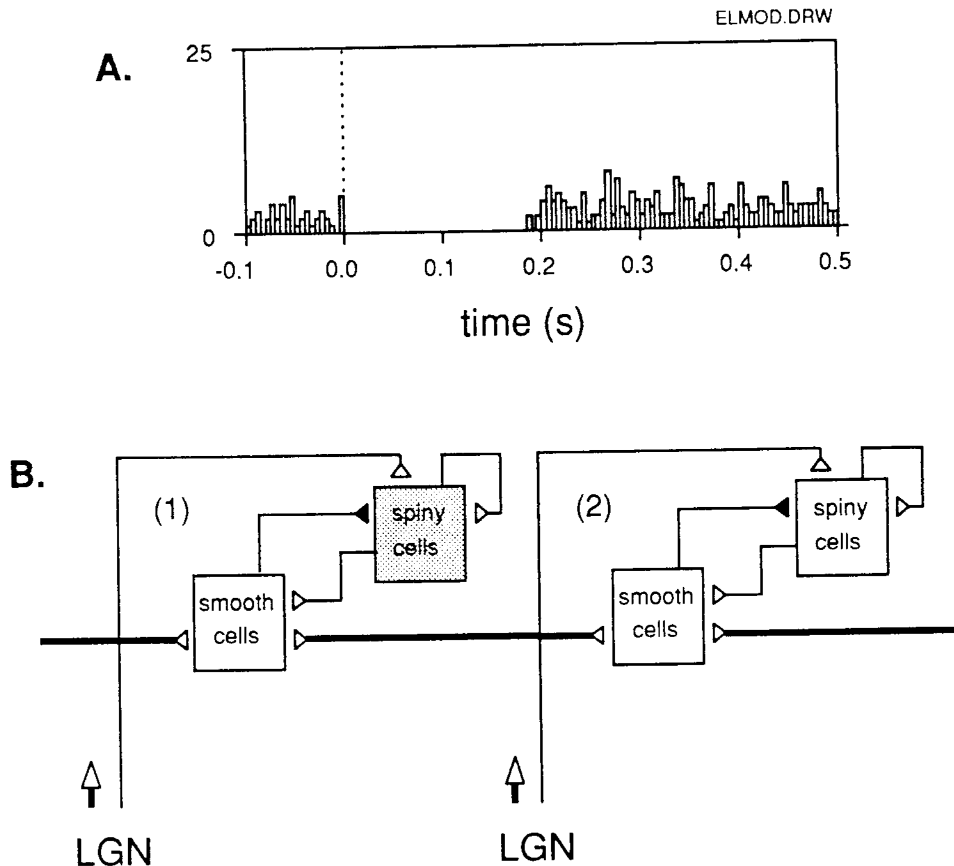


Fig. 7.3. (**A**) Typical extracellular cortical response to electrical stimulation. (**B**) Hypothetical model used to explain the electrically evoked *in vivo* sustained inhibitory response. The synchronous activation of the LGN afferents ensures that there is no preferential activation of the spiny cell population. The LGN afferents to smooth cells are myelinated right up the boutons (as depicted by the thick solid lines). This ensures that the electrically induced LGN activation will always excite the smooth cell population first, before the spiny cell population. The GABAergic inhibitory population thus prevents the spiny neurones from generating a sustained excitation.

It does not necessarily imply that with flash stimulation, a smaller number of geniculo-cortical fibres are activated as compared to that upon electrical stimulation. For the activation of any local cortical microcircuit, the input to that circuit needs to be ordered, as depicted in Fig. 7.2. B. This does not exclude the possibility that other cortical microcircuits could also be activated simultaneously from other retino-geniculate inputs (e.g. module 2 in Fig. 7.2 B). With electrical stimulation, the volume of geniculate tissue stimulated may be smaller than that with natural stimulation, depending to a certain

extent on the stimulus strength and electrode configuration (see Ranck, 1981).

In summary, it is suspected that during strobe-flash stimulation, the spatially ordered input to cortex ensures that initially, only a small fraction of the inhibitory (GABAergic) capacity of cortex is utilised, thus allowing for the visibility of primary and secondary excitatory events. On the other hand, the nature of the electrical pulse stimulus i.e. its synchronous activation of the stimulated fibres, unleashes intracortical inhibition to its full capacity as early as possible, and so prevents the spiny (excitatory) neurones from re-exciting one another to produce a sustained excitatory response.

7.4. Possible explanation for the flash-evoked cortical sensitivity to changes in the level of surround illumination

In chapter 5, it was shown that the temporal profile of the cortical response to flash stimulation could often be modulated by small changes in the intensity of the surround (or background) illumination. Most evident, was the emergence of a brief (40-60 ms) secondary inhibitory phase at the lowest level of background illumination i.e. in complete darkness (0 lux). A possible explanation for this moderate sensitivity, comes from the combination of analysing the changes in the receptive field structure of retinal ganglion cells with changing background illumination and a knowledge of the basic organisation of the excitatory and inhibitory cortical population (as depicted in Fig. 7.2 B). Barlow *et al* (1957) showed that the inhibitory surrounds of ON-like retinal ganglion cells (in cats) disappeared when the background illumination was at 0 lux i.e. complete darkness. This implies that there will be a greater net excitation arriving at the LGN because the inhibitory outputs from the retina would now be suppressed. Consequently, a greater net excitation will arrive at cortex. As pointed out in section 7.3 (Fig. 7.2 B), we assume that with natural stimulation, there is a preferential activation of the LGN afferents that synapse on the spiny cell population. With zero background illumination, the same cortical circuit (as that shown in Fig. 7.2 B) will be engaged, but there will be a greater net excitation arriving at the spiny population. This in turn could activate the smooth inhibitory population earlier than would normally occur with higher levels of background illumination. This implies that for just 40-60 ms after the initial cortical excitation, the smooth cells prevent the spiny cells from firing. The degree of this flash-evoked inhibition clearly does not match the sustained electrically

evoked inhibition. This is the only clue we have at present, in providing an explanation for the moderate increase of the flash-evoked secondary inhibitory phase in cortical neurones upon shifting from post-zero to a zero (complete darkness) level of background illumination.

7.5. Origin and mechanism of the flash-evoked inhibition in LGN (thalamic) neurones

The origin and mechanism of the flash-evoked LGN inhibition is not directly relevant to the overall objectives of this study i.e. understanding *cortical* processing. The mechanisms that might be responsible for the LGN inhibition has been discussed in detail in chapter 2. Briefly, there is good anatomical and moderate physiological evidence to suggest that the extensive inhibitory terminations on geniculate neurones that arise from the reticular nucleus of the thalamus (RNT), are activated by striate (primary visual) and extrastriate (secondary visual) cortical neurones (Sherman & Koch, 1990; Crick, 1984; Murphy & Sillito, 1987; Singer, 1977). Some studies have provided evidence for the existence of intrinsic inhibitory mechanisms in generating sustained inhibition in geniculate neurones (Jahnsen & Llinas, 1984; Sherman & Koch, 1990; Crunelli & Leresche, 1991). In relation to the present study, the sustained flash-evoked cortical excitation (e.g. Fig. 7.1 B) might very well account for part of the sustained quiescence of the flash-evoked LGN response (e.g. Fig. 7.1 A). This is because the cortex can indirectly (via the RNT) exert strong negative feedback on thalamic neurones (see chapter 2).

7.6. Direction for future work

The cortical data presented in this study (chapter 5) were found to be consistent with two different types of anaesthetics (equithesin & saffan) and two different routes of one of these anaesthetic agents (equithesin). As far as the response to electrical stimulation was concerned, it was shown that the sustained inhibitory response was so stereotypical that it could be reproduced not only in visually responsive cortical units but also in cortical units that were completely unresponsive to natural stimuli. Further, this electrically-evoked sustained inhibition in cortex has been found in other studies using cats (Berman, 1991; Douglas & Martin, 1991). Transient natural i.e. strobe-flash stimuli evoke cortical responses that are unique to each tested unit. But the consistent finding in all of these units is the sustained re-excitation. The temporal form of this sustained re-excitation is slightly different in different neurones, and this might be partly dependent on the level of background illumination. That it *exists* for hundreds of milliseconds in the majority of tested neurones is the important finding. That such sustained re-excitatory events are absent in LGN neurones, which feed the cortex with the visually encoded information arriving from the retina, was confirmed with two different routes of equithesin administration i.e. the intraperitoneal and intravenous routes. The primary advantage of intravenous anaesthesia is that it allows for long-term stability of the level of anaesthesia (see chapter 4). As highlighted in chapter 2, previous studies (Fukuda *et al*, 1973 & 1975) using yet another anaesthetic (urethane), have shown that the flash-evoked LGN response (in rats) is also free of sustained excitatory events that extend to hundreds of milliseconds. Consequently, it is most unlikely that our findings are attributed to artefacts of a particular type of anaesthetic.

Because the data presented in this study might have important implications for many theories of cortical processing (see chapter 5 and section 7.2 & 7.3), our experiments should be repeated in unrestrained alert animals. Techniques are available whereby recording electrodes can be chronically implanted in the central nervous system of trained animals without them experiencing any pain (Lemon, 1984). However, many technical and ethical issues associated with these procedures have thus far, ensured that single unit recording in fully anaesthetised animals (as was the case in this study) is still the method of choice. There is presently no need to shift from extracellular to intracellular recording. The primary advantage of intracellular recording would be that it would allow for the visibility of sub-

threshold membrane conductances from which one could assess the degree to which inhibition is mediated via hyperpolarising or shunting mechanisms. Considering that only a small fraction of the inhibitory capacity of cortex is utilised during natural stimulation, this effort would be of little value to our immediate goals of understanding cortical processing. Finally, it would be interesting to test if sustained re-excitatory events to transient natural stimuli are a common feature of cortical neurones in "higher" mammals like cats and monkeys. Only then, should we begin to address the mechanisms of specific neuronal computations like direction and orientation selectivity in visual cortical neurones.

Chapter 8

CONCLUSIONS

The use of transient stimuli in this study have elicited dynamics of cortical processing that previous studies using sustained stimuli could not.

Transient electrical stimulation of the LGN and transient strobe-flash stimulation of the retina evoked cortical responses that were distinctly different. Electrical stimulation evoked a protracted period of inhibition (197 ± 61 ms) followed by a late rebound (excitatory) discharge. By contrast, flash stimulation evoked a prominent initial excitatory phase followed by sustained secondary excitatory events that continued for 200-300 ms. The flash-evoked secondary phase of cortical re-excitation was independent of the type of anaesthetic used and only marginally dependent on the level of background illumination. The important implication of the difference in the flash and electrically evoked cortical response is that even though substantial inhibition is available to the cortex, only a small fraction of this inhibitory capacity is utilised during natural stimulation.

We excluded the possibility that the cortical phase of re-excitation was as a result of a sustained input from the LGN. Neurones of the LGN, which feed the cortex with visually encoded information from the retina, did not display flash-evoked sustained excitatory events, irrespective of the level of background illumination. So the late phase of re-excitation in cortex had no corresponding thalamic input since LGN neurones were completely silent while cortical neurones continued to process the flash stimulus. This difference in the LGN and cortical flash-evoked response represents a non-linear transformation of visually encoded information from the thalamus to the visual cortex. These findings emphasize the independent role of the cortex in computing the response to visual stimuli, and cast doubt on traditional theories that have emphasized the role of the thalamus in shaping cortical responses.

REFERENCES

Anderson, J.A. & Rosenfeld, E. (1988). *Neurocomputing: Foundations of Research*. MIT Press. Cambridge, Massachusetts.

Barlow, H.B.; Fitzhugh, R. & Kuffler, S.W. (1957). Change of organization in the receptive fields of the cat's retina during dark adaptation. *Journal of Physiology* **137**, 338-354.

Barlow, H.B. (1972). Single units and sensation: A neuron doctrine for perceptual psychology. *Perception* **1**, 371-394.

Barlow, H.B. (1982). David Hubel and Torsten Wiesel. Their contributions towards understanding the primary visual cortex. *Trends in Neurosciences* **5**, 145-152.

Barlow, H.B. (1985). Cerebral cortex as a model builder. In: *Models of the Visual Cortex*, Rose, D. & Dobson, V.G. ed., John Wiley & sons Ltd. New York. pp 37-46.

Berman, N.J. (1991). Aspects of inhibition in the visual cortex. Ph.d. thesis. Lincoln College, Oxford University.

Berman, N.J.; Douglas, R.J. & Martin, K.A.C. (1989). The conductances associated with inhibitory postsynaptic potentials are larger in visual cortical neurones *in vitro* than in similar neurones in intact anaesthetized rats. *Journal of Physiology* **418**, 107P.

Berman, N.J.; Douglas, R.J. & Martin, K.A.C. (1992). GABA-mediated inhibition in the neural networks of visual cortex. *Progress in Brain Research* **90**, 443-476.

Beaulieu, C. & Colonnier, M. (1983). The number of neurones in different laminae of the binocular and monocular regions of area 17 in the cat. *The Journal of Comparative Neurology* **217**, 337-344.

Burne, R.A.; Parnavelas, J.G. & Lin, C.S. (1984). Response properties of neurones in the visual cortex of the rat. *Experimental Brain Research* **53**, 374-383.

Carpenter, R.H.S. (1990). *Neurophysiology*. Edward Arnold Press. London.

Churchland, P.M. & Churchland, P.S. (1990). Could a machine think? *Scientific American* **262**, 26-31.

Clark, D.L. & Rosner, B.S. (1973). Neurophysiologic effects of general anaesthetics. *Anaesthesiology* **38**, 564-582.

Crabtree, J.W. & Killackey, H.P. (1989). The topographic organization and axis of projection within the visual sector of the rabbit's thalamic reticular nucleus. *European Journal of Neuroscience* **1**, 94-109.

Creutzfeldt, O.D. (1977). Generality of the functional structure of the neocortex. *Naturwissenschaften* **64**, 507-515.

Creutzfeldt, O.; Rosina, A.; Ito, M. & Probst, W. (1969). Visual evoked response of single cells and of the EEG in primary visual area of the cat. *Journal of Neurophysiology* **32**, 127-137.

Crick, F. (1984). Functions of the thalamic reticular complex: The searchlight hypothesis. *National Academy of Sciences Proceedings.* USA **81**, 4586-4590.

Crick, F. (1989). The recent excitement about neural networks. *Nature* **337**, 129-132.

Crunelli, V. & Leresche, N. (1991). A role for $GABA_B$ receptors in excitation and inhibition of thalamocortical cells. *Trends in Neurosciences* **14**, 16-21.

Dehay, C., Douglas, R.J., Martin, K.A.C., & Nelson, C. (1991). Excitation by geniculocortical synapses is not vetoed at the level of dendritic spines in cat visual cortex. *Journal of Physiology* **440**, 723-734.

Douglas, R.J. & Martin, K.A.C. (1990). Neocortex. In: *The Synaptic Organisation of the Brain*, Shepherd, G. ed., Oxford University Press. New York. pp 389-438.

Douglas, R.J. & Martin, K.A.C. (1991). A functional microcircuit for cat visual cortex. *Journal of Physiology* **440**, 735-769.

Douglas, R.J.; Martin, K.A.C. & Whitteridge, D. (1989). A canonical microcircuit for neocortex. *Neural Computation* **1**, 480-488.

Douglas, R.J.; Martin, K.A.C. & Whitteridge, D. (1991). An intracellular analysis of the visual responses of neurones in cat visual cortex. *Journal of Physiology* **440**, 659-696.

Eccles, J.C. (1984). The cerebral neocortex. A theory of its operation. In: *Cerebral Cortex*, Vol. 2, *Functional Properties of Cortical Cells*, Jones, E.G. & Peters, A. eds., Plenum Press, New York. pp 1-36.

Engel, A.K.; Konig, P.; Gray, C.M. & Singer. W. (1990). Stimulus-dependent neuronal oscillations in cat visual cortex: Inter-columnar interaction as

determined by cross-correlation analysis. *European Journal of Neuroscience* **2**, 588-606.

Espinoza, S.G. & Thomas, H.C. (1983). Retinotopic organisation of striate and extrastriate visual cortex in the hooded rat. *Brain Research* **272**, 137-144.

Ferster, D. (1988). Spatially opponent excitation and inhibition in simple cells of the cat visual cortex. *The Journal of Neuroscience* **8**, 1172-1180.

Freund, T,F.; Martin, K.A.C.; Somogyi, P. & Whitteridge, D. (1985). Innervation of cat visual areas 17 and 18 by physiologically identified X and Y type thalamic afferents. II. Identification of postsynaptic targets by GABA immunocytochemistry and Golgi impregnation. *The Journal of Comparative Neurology* **242**, 275-291.

Frisby, J.P. (1979). *Seeing: Illusion, Brain and Mind.* Oxford University Press, New York.

Fukuda, Y.; Sugitani, M. & Iwama, K. (1973). Flash-evoked responses of two types of principal cells of the rat lateral geniculate body. *Brain Research* **57**, 208-212.

Fukuda, Y.; Sugitani, M. & Kim, Y. (1975). A further study on fast and slow principal cells of rat lateral geniculate body: An analysis of flash-evoked responses. *Tohoku Journal of Experimental Medicine* **115**, 33-45.

Giuffrida, R. & Rustioni, A. (1988). Glutamate and aspartate immunoreactivity in corticothalamic neurones of rats. In: *Cellular Thalamic Mechanisms*, Macchi, G.; Bentivoglio, M. & Spreafico, R. eds., Elsevier. New York. pp 311-320.

Gray, C.M.; Engel, A.K.; Konig, P. & Singer, W. (1990). Stimulus-dependent neuronal oscillations in cat visual cortex: Receptive field properties and feature dependence. *European Journal of Neuroscience* **2**, 607-619.

Green, C.J. (1979). *Laboratory Animal Handbooks 8: Animal Anaesthesia.* Laboratory Animals Ltd, London.

Green, C.J.; Halsey, M.J.; Precious, S. & Wardley-Smith, B. (1978). Alphaxalone-alphadolone anaesthesia in laboratory animals. *Laboratory Animals* **12**, 85-89.

Hubel, D.H. & Wiesel, T.N. (1959). Receptive fields of single neurones in the cat's striate cortex. *Journal of Physiology* **148**, 574-591.

Hubel, D.H. & Wiesel, T.N. (1961). Integrative action in the cat's lateral geniculate body. *Journal of Physiology* **155**, 385-398.

Hubel, D.H. & Wiesel, T.N. (1962). Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *Journal of Physiology* **160**, 106-154.

Hubel, D.H. (1988). *Eye, Brain & Vision*. Scientific American Library. New York.

Hull, E.M. (1968). Corticofugal influence in the macaque lateral geniculate nucleus. *Vision Research* **8**, 1285-1298.

Humphrey, A.L. & Weller, R.E. (1988). Functionally distinct groups of X-cells in the lateral geniculate nucleus of the cat. *The Journal of Comparative Neurology* **268**, 429-447.

Jahnsen, H. & Llinas, R. (1984). Electrophysiological properties of guinea-pig thalamic neurones: An *in vitro* study. *Journal of Physiology* **349**, 205-226.

Kalil, R.E. & Chase, R. (1970). Corticofugal influence on the activity of lateral genciulate neurones in the cat. *Journal of Neurophysiology* **33**, 459-474.

Kulli, J. & Koch, C. (1991). Does anaesthesia cause loss of consciousness? *Trends in Neurosciences* **14**, 6-10.

Kunt, U. & Creutzfeldt, O.D. (1971). Decreased post synaptic inhibition in the visual cortex during flicker stimulation. *Electroencephalography and Clinical Neurophysiology* **30**, 79-82.

Lehky, S.R. & Sejnowski, T.J. (1988). Network model of shape-from-shading: Neural function arises from both receptive and projective fields. *Nature* **333**, 452-454.

Lemon, R. (1984). Theoretical background to recording and stimulation in conscious animals. In: *Methods for neuronal recording in conscious animals*, Smith, A.D. ed., John Wiley & Sons. New York. pp 17-42.

Lorente de No, R. (1949). Cerebral cortex: Architecture, intracortical connections, motor projections. In: *Physiology of the Nervous System*, Fulton, J.F. ed., Oxford University Press, New York. pp 288-315.

Mahowald, M. & Douglas, R. (1991). A silicon neurone. *Nature* **354**, 515-518.

Mahowald, M. & Mead, C. (1991). The silicon retina. *Scientific American* **264**, 40-46.

Martin, K.A.C. & Perry, V.H. (1988). On seeing a butterfly: the physiology of vision. *Scientific Progress at Oxford* **72**, 259-280.

Martin, K.A.C. (1988a). The welcome prize lecture. From single cells to simple circuits in the cerebral cortex. *Quarterly Journal of Experimental Physiology* **73**, 637-702.

Martin, K.A.C. (1988b). From enzymes to visual perception : a bridge too far ? *Trends in Neurosciences* **11**, 380-387.

Martin, K.A.C. (1988c). The lateral geniculate nucleus strikes back. *Trends in Neurosciences* **11**, 192-194.

Mason, C. & Kandel, E.R. (1991). Central visual pathways. In: *Principles of Neural Science*, Kandel, E.R.; Schwartz, J.H. & Jessell, T.M. eds., Elsevier. New York. pp 420-439.

Matthews, M.A. (1973). Death of the central neurone: An electron microscopic study of thalamic retrograde degeneration following cortical ablation. *Journal of Neurocytology* **2**, 265-288.

McCormick, D.A. (1990). Membrane properties and neurotransmitter actions. In: *The Synaptic Organisation of the Brain*, Shepherd, G. ed., Oxford University Press. New York. pp 32-66.

Minsky, M. & Papert, P. (1969). *Perceptrons*. MIT Press. Cambridge, Massachusetts.

Moody, C.I. & Sillito, A.M. (1988). The role of the *N*-methyl-D-aspartate (NMDA) receptor in the transmission of visual information in the feline dorsal lateral geniculate nucleus (dLGN). *Journal of Physiology* **396**, 62P.

Murphy, P.C. & Sillito, A.M. (1987). Corticofugal feedback influences the generation of length tuning in the visual pathway. *Nature* **329**, 727-729.

Nielsen, J.A. & Moore, K.E. (1982). Effects of chloral hydrate, gamma-butyrolactone, and equithesin on the efflux of dopamine and 5-hydroxytryptamine metabolites into cerebroventricular perfusates of rats. *Journal of Neurochemistry* **39**, 235-238.

Paxinos, G. & Watson, C. (1986). *The Rat Brain in Stereotaxic Coordinates*. Academic Press. New York.

Perrett, D.I.; Mistlin, A.J. & Chitty, A.J. (1987). Visual neurones responsive to faces. *Trends in Neurosciences* **10**, 358-364.

Peters, A. (1985). Neuronal composition and circuitry of rat visual cortex. In: *Models of the Visual Cortex*, Rose, D. & Dobson, V.G. ed., John Wiley & Sons Ltd, New York. pp 492-503.

Peters, A.; Kara, D.A. & Harriman, K.M. (1985). The neuronal composition of area 17 of rat visual cortex. III. Numerical considerations. *The Journal of Comparative Neurology* **238**, 263-274.

Polyak, S. (1957). *The Vertebrate Visual System*, Kluver, H. ed., University of Chicago Press. Chicago. pp 451-459.

Powell, T.P.S. (1981). Certain aspects of the intrinsic organisation of the cerebral cortex. In: *Brain Mechanisms and Perceptual Awareness*, Pompeiano, O. & Ajmone-Marsan, C. eds., Raven Press, New York. pp 1-19.

Ranck, J.B. (1981). Extracellular stimulation. In: *Electrical Stimulation Research Techniques*. Patterson, M.M. & Kesner, R.P. eds., Academic Press. New York. pp 1-36.

Rauschecker, J.P. & Singer, W. (1981). The effects of early visual experience on the cat's visual cortex and their possible explanation by Hebb synapses. *Journal of Physiology* **310**, 215-239.

Richmond, B.J.; Optican, L.M. & Spitzer, H. (1990). Temporal encoding of two-dimensional patterns by single units in primate primary visual cortex. I. Stimulus-response relations. *Journal of Neurophysiology* **64**, 351-369.

Sefton, A.J. & Dreher, B. (1985). Visual system. In: *The Rat Nervous System*. Paxinos, G. ed., Academic Press. Australia. pp 169-221.

Sejnowski, T.J. & Rosenberg, C.R. (1988). NETtalk: a parallel network that learns to read aloud. In: *Neurocomputing: Foundations of Research*, Anderson, J.A. & Rosenfeld, E. eds., MIT Press. Cambridge, Massachusetts. pp 663-672.

Shepherd, G.M. & Koch, C. (1990). Introduction to Synaptic Circuits. In: *The Synaptic Organisation of the Brain*, Shepherd, G. ed., Oxford University Press. New York. pp 3-31.

Sherman, S.M. & Koch, C. (1986). The control of retinogeniculate transmission in the mammalian lateral geniculate nucleus. *Experimental Brain Research* **63**, 1-20.

Sherman, S.M. & Koch, C. (1990). Thalamus. In: *The Synaptic Organisation of the Brain*, Shepherd, G.M. ed., Oxford University Press. New York. pp 246-278.

Sherman, S.M. (1985a). Functional organisation of the W, X and Y cell pathways in the cat : A review and hypothesis. In: *Progress in Psychological and Physiological Psychology, VII*. Sprague, J.M. & Epstein, A.W. eds., Academic Press, New York. pp 233-314.

Sherman, S.M. (1985b). W, X and Y cell pathways in the cat : A model for visual function. In: *Models of the Visual Cortex*, Rose, D. & Dobson, V.G. ed., John Wiley & Sons Ltd, New York. pp 71-84.

Sillito, A.M.; Kemp, J.A.; Wilson, J.A. & Berardi, N. (1980). A re-evaluation of the mechanisms underlying simple cell orientation selectivity. *Brain Research* **194**, 517-520.

Simons, D.J. & Carvell, G.E. (1989). Thalamocortical response transformation in the rat vibrissa/barrel system. *Journal of Neurophysiology* **61**, 311-330.

Singer, W. (1977). Control of thalamic transmission by corticofugal and ascending reticular pathways in the visual system. *Physiological Reviews* **57**, 386-420.

Somogyi, P. (1989). Synaptic organisation of GABAergic neurones and $GABA_A$ receptors in the lateral geniculate nucleus and visual cortex. In: *Neural mechanisms of visual perception*, Lam, D.K.-T. & Gilbert, C.D. ed., Portfolio Publishing Company. Houston. pp 35-62.

Somogyi, P. & Freund, T.F. (1989). Immunocytochemistry and synaptic relationships of physiologically characterized HRP-filled neurones. In: *Neuroanatomical Tract-Tracing Methods 2: Recent Progress*, Heimer, L. & Zaborszky, L. eds., Plenum Press. New York. pp 239-264.

Stevens, C.F. (1989). How cortical interconnectedness varies with network size. *Neural Computation* **1**, 473-479.

Sumitomo, I.; Sugitani, M. & Iwama, K. (1977). Disinhibition of perigeniculate reticular neurones following chronic ablation of the visual cortex in rats. *Tohoku Journal of experimental Medicine* **122**, 321-329.

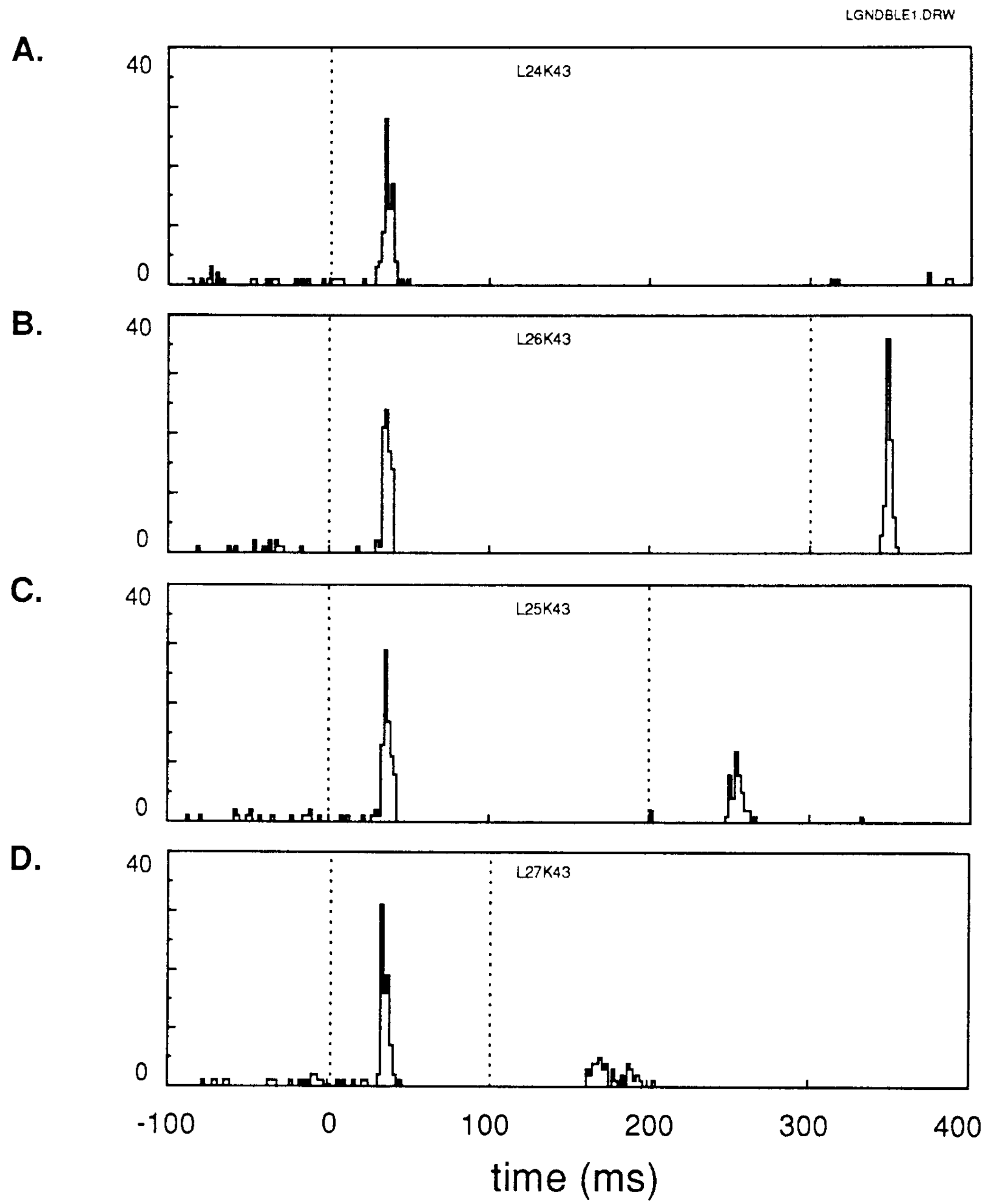
White, E.L. (1989). *Cortical Circuits : Synaptic Organisation of the Cerebral Cortex. Structure, Function, and Theory.* Birkhauser, Boston.

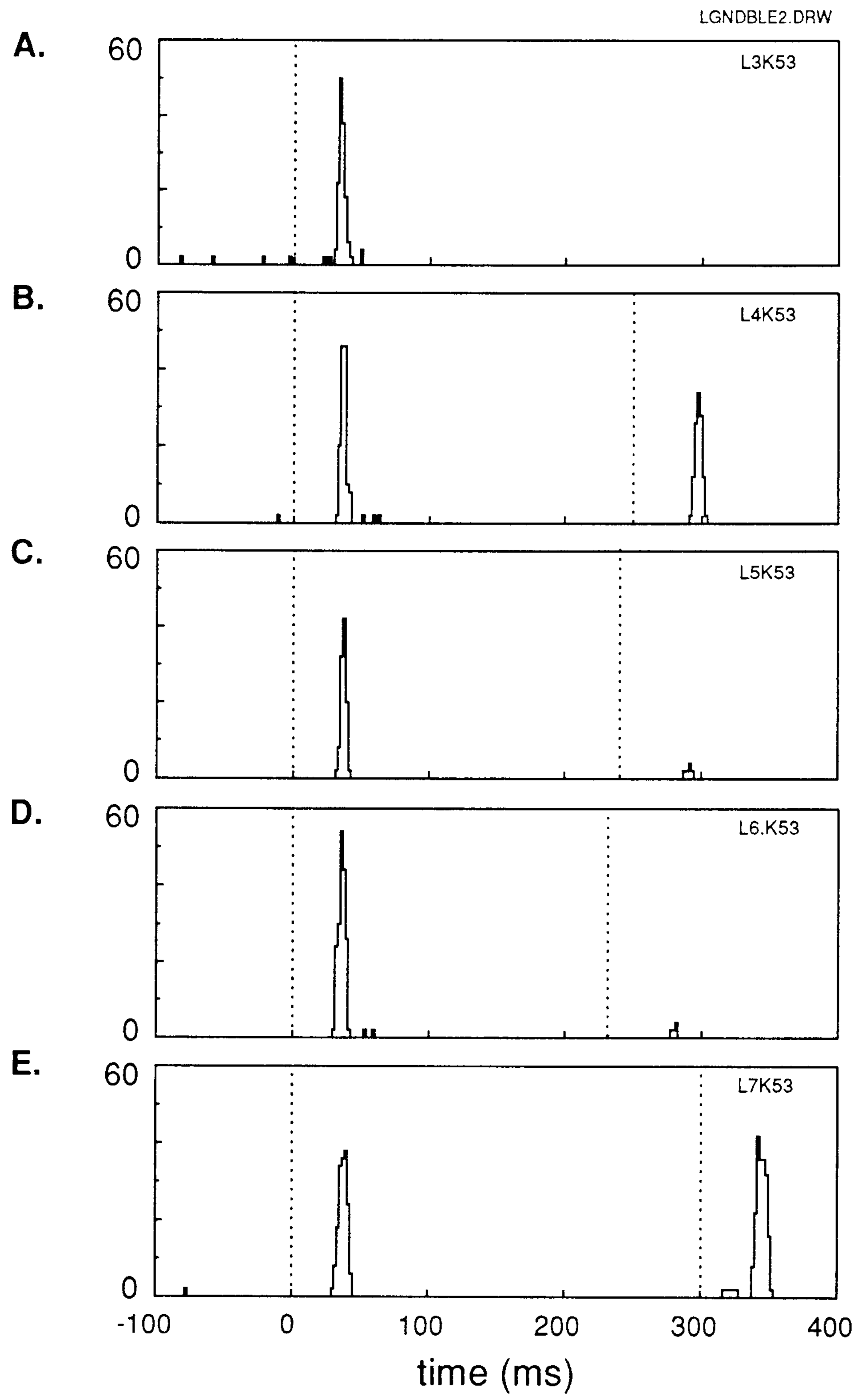
Zornetzer, S.F.; Davis, J.L. & Lau, C. (1990). *An Introduction to Neural and Electronic Networks*. Academic Press. New York.

APPENDIX

Appendix 1: LGN response to paired flash stimulation

On the following two pages are two examples of LGN neuronal responses to paired flash stimulation. The methods and form of these histograms are identical to those used for the single flash responses shown in chapter 6. These paired responses are included in this appendix as they were done by myself in an earlier (Hons) project. They are included purely for reference purposes for the author. The significance of the results of these paired responses are that a protracted period of inhibition follows the early peak. The second of the paired flash stimuli (indicated by the dotted lines) produces a poor (Fig. LGNDBLE1.DRW) or no response (Fig. LGNDBLE2.DRW) if that second flash is applied within the inhibitory period of the response. These findings exclude the necessity to record the cortical responses to paired flash stimuli that are applied within 200 ms of each other.


LGNDBLE1.DRW
A.
B.
C.
D.
40
0
L24K43
L26K43
L25K43
L27K43
-100
0
100
200
300
400
time (ms)



LGNDBLE2.DRW
A.
B.
C.
D.
E.
60
0
L3K53
L4K53
L5K53
L6.K53
L7K53
-100
0
100
200
300
400
time (ms)

Appendix 2: Computer programs used for data analysis

Prog.1: Cxdump.txt. Used for assimilating and printing raw spike2 raster and PSTH files.

```
VAR err
VAR num
VAR fName$
VAR fnum$
VAR fRoot$
fRoot$:="lgn47h" ;
num:=0
REPEAT
IF num<10 ; ;
PRINT 5 fnum$ "%1.0d" num ;
fName$:=fRoot$+"0"+fnum$+".SMR" ;
ELSE ;
PRINT 5 fnum$ "%2.0d" num ;
fName$:=fRoot$+fnum$+".SMR" ;
ENDIF ;
FILE fName$ err ;
IF err=0 ;
 CLEAR ; VIEW 1 ; WINDOW 0 0 50 40 ;
 VIEW 2 ; WINDOW 50 0 100 40 ;
 VIEW 3 ; WINDOW 0 40 100 80 ;
 VIEW 1 ; DRAW 0 MAXTIME ;
 VIEW 2 ; OFF ALL ; OFF XAXIS ; RASTER 4 1 ; DRAW 0 MAXTIME
;
 VIEW 1 ; SETPSTH 3 1 125 0.005 0 4 ; PROCESS 0 MAXTIME ;
 VIEW 3 ; YRANGE 1 0 20 ; DRAW ;
 VIEW 1 ; SETPSTH 3 2 125 0.005 0 4 ; PROCESS 0 MAXTIME ;
 VIEW 3 ; ON OVERDRAW ; COLOUR 9 12 ; DRAW ; OFF
OVERDRAW ; COLOUR 9 11 ;
' VIEW 1 ; SETPSTH 3 3 125 0.005 0 4 ; PROCESS 0 MAXTIME ;
 VIEW 3 ; ON OVERDRAW ; COLOUR 9 9 ; DRAW ; OFF
OVERDRAW ; COLOUR 9 11 ;
 MOVETO 50 50 ; PRINT 1 fName$ ;
 MOVETO 25 9 ; PRINT 1 "%4d" SWEEPS ;
 MOVETO 0 85 ;
 FOR i:=1 5
  PRINT 1 "%s" FILECOM[i] ; NEXT i ;
' INTERACT ; 'edit this line out when automatic screen dump required
 SCRNDUMP ;
 num:=num+1 ;
 ENDIF ;
UNTIL err>0 ;
END ;
```

Prog. 2: Cxsum.txt. Used to export final Spike2 PSTH examples to Lotus Freelance Graphics for Postscript printing.

```
VAR period ; period:=0.6 ;
VAR bsz ; bsz:=0.005 ;
VAR bins ; bins:=period/bsz ;
VAR t0 ; t0:=0 ;
VAR t1 ; t1:=MAXTIME ;
VAR ymax ; ymax:=20 ;
VAR fName$ ;

CLEAR ; VIEW 1 ; WINDOW 0 0 50 30 ;
VIEW 2 ; WINDOW 0 30 50 60 ;
VIEW 3 ; WINDOW 50 0 100 30 ; OFF TRAM FRAME ;
VIEW 4 ; WINDOW 50 30 100 60 ; OFF TRAM FRAME ;
VIEW 5 ; WINDOW 50 60 100 90 ; OFF TRAM FRAME ;
VIEW 6 ; WINDOW 0 60 50 90 ; OFF TRAM FRAME ;

VIEW 1 ; DRAW 0 MAXTIME ; t1:=MAXTIME ;
VIEW 2 ; OFF ALL ; RASTER 4 1 ; YRANGE 4 0 period ; DRAW t0 t1 ;

VIEW 1 ; SETPSTH 3 1 bins bsz 0 4 ; PROCESS t0 t1 ; VIEW 3 ;
YRANGE 1 0 ymax ; DRAW ;
VIEW 1 ; SETPSTH 4 2 bins bsz 0 4 ; PROCESS t0 t1 ; VIEW 4 ;
YRANGE 1 0 ymax ; DRAW ;
VIEW 1 ; SETPSTH 5 3 bins bsz 0 4 ; PROCESS t0 t1 ; VIEW 5 ;
YRANGE 1 0 ymax ; DRAW ;
VIEW 1 ; SETPSTH 6 1 100 0.0005 0.01 2 ; PROCESS t0 t1 ; VIEW 6 ;
YRANGE 1 0 ymax ; DRAW ;

MOVETO 75 5  ; PRINT 1 "%4d sweeps"  SWEEPS ;
MOVETO 75 35 ; PRINT 1 "flash"
MOVETO 75 65 ; PRINT 1 "elec"
FKEY 0 ;
FKEY 1 9 PlotPic "PlotPic"
 REPEAT
 FKEY 5
 UNTIL ESCAPE
END ;

PROC PlotPic
VIEW 4
INPUTSTR fName$ "PIC plotfile" 8
PLOTTO fName$ 1
MOVETO 75 5 ; PRINT 4 fName$ ;
VIEW 3 ; PLOT 1 bins ; MOVETO 85 5 ; PRINT 4 "%4d sweeps"
SWEEPS ;
'VIEW 4 ; PLOT 1 bins ; MOVETO 75 35 ; PRINT 4 "flash";
'VIEW 5 ; PLOT 1 bins ; MOVETO 75 65 ; PRINT 4 "elec" ;
'VIEW 6 ; PLOT 1 100
RETURN
```