



**CONNECTIVITY AND RESPONSE CHARACTERISTICS OF THE CELLS
OF AREA 21A IN THE CAT**

I declare that all the research presented in this thesis is original work, and, to the best of my knowledge, has not been published or submitted for publication elsewhere.

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

by

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Declaration

I declare that all the research presented in this thesis is my own work and, to the best of my knowledge, this thesis contains no material previously published or written by another person. This thesis contains no material which has been submitted or accepted for the award of any other degree or diploma in any university.



(Brian Wimborne)

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CONTENTS		PAGE
CHAPTER 1: GENERAL INTRODUCTION		1
Cerebral Cortex		2
Association Cortex		3
Visual Association Cortex		7
Visual Cortex in the Cat		11
Area 21a of the Cat's Association Cortex		13
Aims and Synopsis of the Research		17
CHAPTER 2: THALAMIC CONNECTIONS WITH AREA 21A		19
INTRODUCTION		20
Serial and Parallel Pathways		20
Hierarchical and Parallel Processing in the Cat		24
Area 21a in the Cat		26
MATERIALS AND METHODS		28
Animal Preparation		28
Injection of Tracer		28
Perfusion and Fixation		30
Preparation of Histological Sections		31
RESULTS		33
HRP-WGA Labelling in the Thalamus		33
(1) <i>LPI</i>		33
(2) <i>Pulvinar</i>		34
(3) <i>LGNd</i>		34
(4) <i>LGNv</i>		35

LPI Projections to Area 21a and the Distribution of AChE	36
Double Fluorescent Labelling in the LPI	37
Biocytin Labelling in the Thalamus	39
(1) LPI	40
(2) Pulvinar	41
(3) LGNd	42
(4) LGNv	42
DISCUSSION	44
Relative Strength of Thalamic Projections to Area 21a	45
(1) LPI	45
(2) Pulvinar	47
(3) LGNd	49
(4) LGNv	50
Terminals from Area 21a Projections to Different Thalamic Nuclei	51
Overview	51
Conclusion	52
CHAPTER 3: CORTICAL CONNECTIONS WITH AREA 21A	53
INTRODUCTION	54
MATERIALS AND METHODS	57
Animal Preparation	57
Perfusion and Fixation	57
Preparation of Histological Sections	58
RESULTS	59
HRP-WGA Labelling	59
Connections with the Ipsilateral Cortex	59
(1) Area 17	60

CHAPTER 4	(2) Area 18	CHARACTERISTICS OF AREA 21A	60
INTRODUC	(3) Area 19		61
MATERIALS	(4) Association Cortex		62
	<i>Animals</i>	<i>Laminar Disposition of Extrinsic Projecting Cells</i>	62
	<i>Methods</i>	<i>Anterograde Transport of HRP-WGA</i>	64
	<i>Results</i>	<i>Summary of HRP-WGA Labelling in the Ipsilateral Cortex</i>	65
		<i>Biocytin Labelling</i>	65
RESULTS	(1) Areas 17 and 18		66
	(2) Other Cortical Areas		67
		<i>Fluorescent Labelling</i>	67
		<i>Overlap of Projections</i>	67
	<i>Overview</i>	<i>Excitatory Regions</i>	68
DISCUSSION	(1) <i>Deposits</i>		70
		<i>Relative Strength of Cortical Projections to Area 21a</i>	70
		<i>Reciprocity of Projections</i>	73
		<i>Laminar Circuitry</i>	74
		<i>Background</i>	74
		<i>Laminar Connectivity with Areas 17, 18 and 19</i>	76
		<i>Laminar Connectivity with the Association Cortex</i>	79
		<i>Duplication of Projections</i>	80
	<i>Overview</i>	<i>Biocytin</i>	80
DISC		<i>Proposed Model of Information Flow</i>	81
		<i>Conclusion</i>	84
		<i>Location of Area 21a's Precursor Cells</i>	85
		<i>Classification of Area 21a's Precursor Cells</i>	88
		<i>Excitatory and Inhibitory Inputs to Area 21a</i>	110
		<i>A Possible Threshold Barrier in Area 21a</i>	111
		<i>Conclusion</i>	113

CHAPTER 4: RESPONSE CHARACTERISTICS OF AREA 21A	85
INTRODUCTION	86
MATERIALS AND METHODS	90
Animal Preparation	90
Visual Stimulation and Recording	91
Reconstruction of Electrode Tracks	93
Perfusion	93
RESULTS	95
Sample	95
Retinotopic Projection	95
Receptive Field Topography	97
(1) Excitatory Regions	97
(i) Disposition	97
(ii) Dimension	98
(2) Inhibitory Regions	100
Properties Influenced by Interactions Within the Receptive Field	101
(1) Summation of Excitation	101
(2) Orientation Specificity	102
(3) Direction Selectivity	104
(4) Velocity Dependence	104
(5) Binocularity	105
DISCUSSION	106
Receptive Field Characteristics of Area 21a Neurons	106
Location of Area 21a's Precursor Cells	107
Classification of Area 21a's Precursor Cells	108
Excitatory and Inhibitory Inputs to Area 21a	110
A Possible Threshold Barrier in Area 21a	111
Conclusion	113

CHAPTER 4: EFFECTS ON AREA 17A FOLLOWING COOLING OF THE

CHAPTER 5: DETECTION OF BINOCULAR ORIENTATION DISPARITY	114
INTRODUCTION	115
MATERIALS AND METHODS	118
Animal Preparation	118
Recording Procedures	118
RESULTS	120
Sample	120
Spatial Disparity	120
Monocular Orientation Tuning Curves	120
(1) Preferred Orientations	120
(2) Sharpness of Orientation Tuning	121
Binocular Orientation Tuning Curves	121
Binocular Orientation Disparity	122
DISCUSSION	123
Differences in Preferred Orientations of Each Eye	123
Specialised Features in Binocular Tuning for	
Orientation and Spatial Disparity	123
Conclusion	125
2. A Comparison of Responses in Area 17 and	
17a with Cooling of the Primary Visual Cortex	126
3. Shape of the Orientation Tuning Curve	131
4. Direction Selectivity	133
5. Velocity Selectivity	143
6. Recovery	144

CHAPTER 6: EFFECTS ON AREA 21A FOLLOWING COOLING OF THE		
DISCUSSION	PRIMARY VISUAL CORTEX	126
INTRODUCTION		127
MATERIALS AND METHODS		129
Animal Preparation		129
Cooling Procedures		129
Visual Stimulation and Recording Procedures		130
RESULTS		132
Sample		132
Experimental Paradigm		132
Temperatures and Temperature Gradients		133
Cooling the Primary Visual Cortex and its Effect on the		137
Neurons of Area 17		135
1. <i>Responsiveness to Visual Stimulation</i>		135
2. <i>Shape of the Orientation Tuning Curve</i>		136
3. <i>Direction Selectivity</i>		137
Cooling the Primary Visual Cortex and its Effect on the		142
Neurons of Area 21a		137
1. <i>Responsiveness to Visual Stimulation</i>		137
2. <i>A Comparison of Responsiveness in Areas 17 and</i>		
<i>21a with Cooling of the Primary Visual Cortex</i>		140
3. <i>Shape of the Orientation Tuning Curve</i>		141
4. <i>Direction Selectivity</i>		143
5. <i>Velocity Selectivity</i>		143
6. <i>Recovery</i>		144

DISCUSSION	145
The Effectiveness of Cooling the Primary Visual Cortex	145
Changes in Responsiveness in Area 17 with Cooling	148
Responsiveness in Area 21a Following Cooling of Area 17	150
Conclusion	153
CHAPTER 7: SUMMARY AND OVERVIEW	154
Aims and Objectives	155
Connectivity and the Place of Area 21a in the Visual Hierarchy	156
(1) <i>Thalamic Connectivity</i>	156
(2) <i>Cortical Connectivity</i>	157
The Response Characteristics of Area 21a Neurons	158
A Possible Role for Area 21a	159
Is there a Primate Homologue for Area 21a?	160
REFERENCES	162

Abbreviations

- ACh.E: Acetylcholinesterase
- AP: Anteroposterior
- ALLS: Anterolateral lateral suprasylvian area
- AMLS: Anteromedial lateral suprasylvian area
- CRZ: Corticorecipient zone
- DLS: Dorsolateral suprasylvian area
- DY: Diamadino yellow
- EVA: Ectosylvian visual area
- FB: Fast blue
- HC: Horsley-Clarke Co-ordinates
- HRP-WGA: Horseradish peroxidase conjugated with wheat germ agglutinin
- LGNd: Dorsal lateral geniculate nucleus
- LGNv: Ventral lateral geniculate nucleus
- LPI: Lateral part of LP-pulvinar complex
- LPm: Medial part of LP-pulvinar complex
- MIN: Medial interlaminar nucleus
- PLLS: Posterolateral lateral suprasylvian area
- PMLS: Posteromedial lateral suprasylvian area
- PRZ: Pretectorecipient zone of the pulvinar
- PS: Posterior suprasylvian area
- RRZ: Retinorecipient zone of the pulvinar
- TMB: Tetramethylbenzidine
- TRZ: Tectorecipient zone
- VLS: Ventral lateral suprasylvian area
- VM: Vertical meridian

Abstract

The most salient findings of this investigation into the connectivity and response characteristics of the cells of area 21a in the cat are set out below.

1. At the level of the thalamus, area 21a has reciprocal connections with the LGNd, pulvinar and LPI and an efferent connection with the LGNv. The projection from the LPI is dense whilst afferents from the LGNd and pulvinar are comparatively weak. This suggests that the LPI may be an important source of signals reaching area 21a. Cells which project from the LPI to areas 17 and 21a are located together. It is possible that a bi-directional information loop exists by which signals pass between area 17 and area 21a via the LPI.

2. At the cortical level, area 21a is reciprocally connected with a number of areas of the primary and association visual cortex. The strongest of these comprise projections from areas 17 and 18 (primary visual cortex) which arise from the supragranular laminae. Projections from area 19 are divided evenly between the supragranular and infragranular laminae, whilst those from areas of the association cortex arise principally from the infragranular laminae. It is postulated that areas 17 and 18 are placed conjointly at a lower level than area 21a in the hierarchy of visual processing and provide feedforward information, whilst area 19 is at a level intermediate between the primary visual cortex and area 21a and may also supply some feedforward data. Regions of the association cortex with which area 21a is connected are believed to reside at a higher level in the hierarchy and to provide feedback information.

3. The receptive fields of the cells of area 21a are centrally located. The cells are binocular, respond with composite ON/OFF firing to a stationary flashing stimulus,

fail to respond to fast-moving stimuli, are not highly selective to direction of movement, exhibit little evidence of inhibition but are often sharply tuned for orientation. Sharpness of orientation tuning despite an apparent absence of significant inhibition may be explained by a model in which a threshold barrier in area 21a excludes lower incoming firing rates. According to this model, orientation inputs from C (complex) cells of the primary visual cortex may be sharpened sufficiently to account for the acuteness of orientation tuning found in area 21a.

4. The binocular nature of the cells and the finding that differences frequently existed between the preferred orientation of each eye, lead to an examination of whether the cells functioned as detectors of binocular orientation disparity. Results, however, did not sustain this hypothesis.

6. The reversible deactivation of areas 17 and 18 was achieved by means of controlled cooling and, at the same time recordings were made in both areas 17 and 21a. This experiment resulted in two significant findings:

i) that area 21a may comprise two populations of cells - one population is wholly dependent for its input on the primary visual cortex whilst the other population appears to receive signals from the primary visual cortex and elsewhere, perhaps area 19;

ii) despite reduced activity of the cells of area 21a when the primary visual cortex was cooled, response features such as direction selectivity and sharpness of orientation tuning were retained almost to the point where the cells firing was extinguished. This indicates that excitatory and inhibitory inputs to area 21a cells were being deactivated by cooling at the same rate.

7. The main conclusions drawn from this study are that area 21a receives its signals principally from C cells of lamina 3 of the primary visual cortex direct and, perhaps, via the LPI. Little, if any, information, however, seems to reach area 21a by a secondary visual pathway via regions of the thalamus. The cells of area 21a play a role in pattern detection in the central region of the visual field and they appear to be crucially involved in the sharpening of orientation inputs.

CHAPTER I

GENERAL INTRODUCTION

Cerebral Cortex

The cerebral cortex is the intricately folded outer layer of the cerebrum making up about 80 per cent of the brain by weight. It is the part of the brain believed to be most directly responsible for consciousness and voluntary activity, with essential roles in perception, motor ability and intellect.

CHAPTER 1

On the evolutionary scale, animals below the level of reptiles do not possess a cerebral cortex, and it is the development of two cerebral hemispheres that is a major distinguishing feature of mammals (Lippold and Lindman, 1951). According to Diamond (1967) the crucial step in the origin of the neocortex was the penetration of sensory fibres from the diencephalon to thalamus into the hitherto olfactory-dominated pallidum and the subsequent increase in the relative size of the non-olfactory cortex.

GENERAL INTRODUCTION

For much of the nineteenth century the doctrine of functional equivalence of the cerebral cortex prevailed. This doctrine was rooted in the ancient belief in a "single, homogeneous and indivisible transcendental soul or mind" (Polyak, 1957) and, as the term implies, it presumed that mental functions were spread equally throughout the cortex. Clinical evidence, however, which challenged this belief was accumulating. In particular, the work of Broca, who proposed that functional plurality applied to the motor and sensory cortex, was especially important (cf. Polyak, 1957). Major support for the idea of functional localization in the cerebral cortex came from Broca's discovery of a motor speech centre which controlled the ability to pronounce words. In addition, Hering and Fehst (cf. Polyak, 1957) demonstrated that electrical stimulation of a dog's frontal lobe resulted in a motor response of the contralateral limbs. These discoveries

Cerebral Cortex

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On the evolutionary scale, animals below the level of reptiles do not possess a cerebral cortex, and it is the development of two cerebral hemispheres that is a major distinguishing feature of the mammalian brain (Bindman and Lippold, 1981). According to Diamond (1967) the crucial step in the origin of the neocortex was the penetration of sensory fibres from the diencephalon or thalamus into the hitherto olfactory-dominated pallium and the consequent increase in the relative size of the non-olfactory cortex.

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together with those of a number of other contemporary scientists brought to an end the widely-held belief in functional equivalence of the entire cortex and paved the way for the discovery that many functions such as memory for visual objects, spoken and written language, as well as the ability to perform skilled movements, etc were localised in the brain. (Paxinos & Watson, 1997)

The cerebral cortex comprises two broad functional regions - motor and sensory cortex. Motor cortex, as the name implies, is concerned with the control of movement whilst sensory cortex is involved with sensation and perception. The sensory cortex is divisible into the primary cortex which receives major direct inputs from the five sensory organs and the secondary or association cortex which is innervated predominantly from the primary cortex. Whilst the division into primary and secondary areas has some rationale behind it in terms of anatomy, histology and electrophysiology, the distinction is seldom equivocal. Histological and anatomical distinctions are often difficult to detect and the functional criteria for separating areas are not always obvious. (Paxinos & Watson, 1997)

Association Cortex

At the time of birth there are five sensory centres in each hemisphere of the human brain that contain myelinated fibres (Polyak, 1957). Each is associated with one of the five primary fissures of the foetal brain, and each corresponds to the terminal area of the principal afferent fibre tracts which arise in one of the peripheral sensory organs. Together, the five centres constitute the primary cortex. The greater part of the cerebral cortex, comprising around two thirds, however, becomes myelinated after birth. About one month after birth regions

adjacent to the sensory centres can be identified and these have been named "marginal zones" by Flechsig (cf Polyak, 1957). "Terminal zones" in turn become identifiable late in the sequence and comprise the frontal lobe, the insula of Reil's "island" lying in the Sylvian pit, much of the temporal lobe and the greater part of the parietal and occipital lobes (Flechsig, cf Polyak, 1957). Flechsig believed that the marginal and terminal zones lack significant afferent and efferent connections with peripheral sensory organs and that they become myelinated at a much later period of development. The marginal and terminal zones comprise the secondary cortex and its connections with peripheral sensory organs were thought to be indirect, by way of association fibres to and from the five primary sensory centres. It was because Flechsig believed the secondary cortex lacked direct input from sensory receptors and was innervated by association fibres only (i.e cortico-cortical connections with the primary cortex), that he designated them "association centres" or "association areas" (*Assoziationzentren*). Flechsig believed these areas to be most highly developed in humans and that they comprised the psychic or thinking centres (*geistige Zentren, Cognitionzentren*) of the brain.

The term, association cortex, has also been used by some investigators to refer to areas where different sensory modalities (e.g. vision and hearing) interact. In this thesis, however, the term is confined solely to sensory cortical areas with connections to the primary cortex.

Although Flechsig's subdivision of the cortex into primary and association areas was valid, some qualification is called for. For example, contrary to his belief that the association cortex is innervated solely by association fibres from the primary cortex, most of the association areas are also connected with sub-cortical areas, particularly the extrageniculate thalamic nuclei and claustrum. According to

Polyak (1957) these sub-cortical nuclei are not the terminals of great afferent sensory tracts of the brain stem and they "apparently transmit impulses of a complex nature, devoid of the character of concrete conscious objectivity". Nevertheless, the retinotopic organisation of many visual association areas seems to be highly dependent on the inputs they receive from thalamic nuclei, particularly the lateral posterior-pulvinar complex (LP-pulvinar complex) (Dreher, 1986). The LP-pulvinar complex has been subdivided into several zones largely on the basis of the origin of its main afferent connections. Although some of these afferents may carry retinal information, they do not form part of the primary visual pathway which reaches the cortex via the dorsal lateral geniculate nucleus (LGNd). The LP-pulvinar complex is reciprocally connected with all areas of the association cortex although many of these connections appear to comprise closed loops.

Of all animals, primates have the greatest volume of association cortex relative to the volume devoted to primary receiving areas (Bindman and Lippold, 1981) and in humans the association areas are relatively large in comparison to other primate species. For this reason, Flechsig considered the association areas to be a specifically human characteristic. As has been stated, the association areas are the last to develop phylogenetically and, according to Polyak (1957), are the material foundation of "man's mastery over other creatures".

In Flechsig's view the functions of the association cortex, as a whole, were twofold. It was to serve as a repository of past impressions (memories) which arrive through the afferent fibres via the sensory centres and it was to participate in the building of complex processes. For instance, the posterior association area, comprising a large part of the parietal, occipital and temporal lobes, would be involved with the integration of the visual, somesthetic and auditory impulses. Its

role would be to retain experiences associated with these three sensory modalities, to integrate them and to perform certain complex actions such as reading and visual understanding of written language, comprehending spoken words or auditory language. In short, Flechsig showed that many mental and sensory faculties, including those that are specifically human such as language, are a functional expression of a complex topographical differentiation and organisation of the cortex.

It is generally accepted that for sensory data to be acted upon and elaborated it must pass from the primary sensory areas to the various association regions. Modern neuronal maps, derived from the use of axonal tracers indicate that this step involves a complex interlinking of numerous cortical and subcortical nuclei. This research has shown that related regions are interconnected in a stepwise fashion which starts from the primary sensory areas such as the visual or somatosensory cortex and progresses through post-Rolandic parasensory areas in the temporal and parietal lobes and multimodal areas (Pandya and Kuypers, 1969; Jones and Powell, 1970; Van Hoesen et al., 1972; Pandya and Seltzer, 1982). This sequential flow of sensory information has been considered an important medium for attention, sensory integration, learning, memory and skilled behaviour.

Despite the fact that the association cortex has long been thought to play a major role in learning, memory and intellect (Flechsig, cf Polyak, 1957; Elliot-Smith, 1910; Campbell, 1905) its function in the earlier phases of sensory perception is not well understood. This may seem somewhat surprising in view of the advances in anatomical, histological and physiological techniques developed over the last three decades. Yet it is a measure of the complexity of neuronal mechanisms that, despite these advances, our understanding of sensory-perceptual processing

is still rudimentary. While Flechsig's description of the association cortex encompasses a number of sensory modalities, it is more usual to discuss it in terms of a single modality.

The sensory areas have been the most thoroughly investigated cortical regions and of these, the visual cortical areas are probably the best understood (Van Essen, 1979). Whilst the striate area or primary visual cortex has been intensely studied, the association cortex is believed to be involved in more abstract levels of visual analysis than that performed by the striate cortex.

Visual Association Cortex

Of the five sensory-perceptual modalities, vision makes perhaps the most important contribution to our understanding of the external world although many might question the statement of Sir Grafton Elliot-Smith (1930) that "Vision is the foundation of intelligence and the chief source of our knowledge." In general terms, research to date indicates that visual perception appears to be the result of an integrative process involving numerous connections between many cortical areas that carry streams of information representing different levels of functional specialisation.

The delineation of the secondary or association visual cortex depends to some degree on establishing the boundaries of the primary visual cortex. Until comparatively recently the term "visual cortex" referred exclusively to the "calcarine" area of Campbell (Diamond, Fitzpatrick and Sprague, 1985). Later this region, which is commonly known as the striate cortex, was named by

Brodmann (1909), Area 17. With increasing evidence of sensory functions for areas of cortex lying rostral to the striate cortex, came the concept of visual association cortex - a modality-specific region concerned with higher visual function. The Australian anatomist, Campbell (1905) referred to this region as the "visuo-psychic" area in contrast to the striate cortex or "visuo-sensory" area, and believed that it received its information exclusively from the striate cortex. He argued, in fact, that "the mere existence of an area contiguous with the striate cortex suggests that it is concerned with sorting out and elaborating the visual impressions produced by the striate cortex" (Ibid).

In assigning separate functions to the "visuo-psychic" and "visuo-sensory" areas, Campbell's reasons seemed well founded. As Flechsig had already pointed-out, the fact that in terms of myelin development the visuo-psychic areas in humans was immature months after the visuo-sensory area had developed, implied that the function of elaborating on simple visual sensations is not required in neonatals. More significantly, the visuo-psychic area did not seem to receive afferents from the visual pathway. In addition, studies of neurological patients indicated a relationship between visual agnosia (inability to recognise and interpret objects) and lesions of the association cortex. Subsequent investigations by comparative anatomists, particularly Elliot-Smith (1910), added weight to the notion that whilst the sensory cortex performed a simple sensation function, the association cortex was involved in the more complex role of interpretation and learning.

Much of the recent work into the role of the visual association cortex has followed

Investigations by Munk (1881) and Schafer (1888) had led to the view that removal of a primary sensory area would result in total or severe sensory deficit. Support for this view came from experiments (Kluver and Bucy, 1937, 1938; Kluver, 1942) in which removal of a monkey's striate cortex resulted in an

inability to discriminate depth, colour, pattern and brightness. Further evidence that lesions of the primary visual cortex result in pattern recognition deficits in higher mammals included results from prosimians (Atencio et al., 1975) and from primates including man (Brindley et al., 1969; Humphrey, 1974). These results appeared to substantiate Campbell's view of the visuo-psychic area being totally dependent on the striate cortex and added weight to the theory of functional cortical localisation although after training it may be possible for higher mammals to discriminate patterns successfully (Atencio et al., 1975; Pasik and Pasik, 1971; Schilder et al., 1972; Weiskrantz et al., 1974).

On the other hand evidence during the last twenty years or so has shown that in more primitive species the visual association cortex may be less dependent on input from the striate cortex. For example, after total removal of the primary visual cortex of *tree shrews* (Killackey, Snyder and Diamond, 1971), *rats* (Spear and Barbas, 1975; Hughes, 1977), *squirrels* (LeVay et al., 1973), *rabbits* (Moore and Murphy, 1976) and *cats* (Spear and Braun, 1969; Doty, 1971; Sprague et al., 1977), total pattern blindness did not result. The obvious inference to be drawn from these findings is that in some species neuronal systems outside the primary visual cortex must have some capability to encode visual shape. According to Berlucchi and Sprague (1981) this capability may be partly intrinsic in the association cortex and partly acquired through a system of neural reorganisation following removal of the primary cortex.

Much of the recent work into the role of the visual association cortex has focussed on monkeys, particularly macaques, where the identification of a large number of cortical visual areas rostral to the primary visual cortex (Zeki, 1969, 1971) plus the uncovering of functional differences between these areas (Zeki, 1973, 1974) resulted in the formulation of the theory of functional specialisation. According

to this theory individual areas of the association cortex are specialised for particular functions such the recognition of motion, colour or form and each area performs a higher level of analysis of its specialised function than was undertaken in the primary visual cortex.

Extracellular recordings made in various areas of the visual association cortex revealed differences in the receptive field properties of neurons and, at first glance, this finding appeared to support the theory of functional specialisation. Nevertheless, there is little solid evidence that each cortical area specialises in a single, unique function or as Van Essen (1985) puts it, that the visual cortex is arranged on a "one area: one function" principle. The middle temporal (MT) area of the macaque monkey, where the majority of cells are selective for both direction and speed of movement (Zeki, 1974; Baker et al., 1981; Maunsell and Van Essen, 1983; Felleman and Kaas, 1984) comes closest to functional specialisation but cells in other areas also appear to be involved with motion detection. Furthermore, area MT may be involved in other visual functions. Evidence that many extrastriate visual areas of the macaque monkey are multifunctional is based on findings that area V-2 cells are selective for wavelength, orientation, spatial disparity and direction; V-3 is implicated in form, depth and motion; and V-4 appears to be involved with the analysis of form, colour and possibly depth.

For these reasons Van Essen (1985) is of the view that it may be more appropriate to think in terms of functional streams rather than functional regions. Anatomical and physiological studies indicate that such streams exist at the earliest levels of the cortex (Maunsell and Newsome, 1987) and it is even possible to identify within the retina, two major processing streams. Approximately 80% of retinal ganglion cells are classed as parvocellular (P) cells which project to the

P layers of the LGNd while around 10% are magnocellular (M) and project to the LGNd M layers (Perry, Oehler and Cowey, 1984). In areas V-1 and V-2 these streams are reorganised into a three-way arrangement which have been designated the P-B, P-I and M streams (DeYoe and Van Essen, 1988) and each stream is associated with two or more types of selectivity (Van Essen, Anderson and Felleman, 1992). For example, there is evidence that, during the early stages of cortical analysis, the P-B stream is involved with analysis of spatial frequency, and wavelength; the P-I stream with orientation, spatial frequency, binocular disparity and wavelength; and the M stream with orientation, spatial frequency, velocity and binocular disparity (Van Essen et al., 1992).

These streams course through the association cortex and the solution to the question of functional specialisation may be to understand the type of transformation which is occurring within each area. Relevant to this issue is the finding that, in general, tuning for individual visual parameters in monkeys do not seem to become sharper as the signals are analysed at successively higher stages of cortical processing (Van Essen, 1985). Whilst this may be attributable to a failure to find subsets of cells responsible for dramatic increases in selectivity, Van Essen (1985) believes it more likely that higher areas are involved with the introduction of selectivity for new parameters or combinations of parameters.

Visual Cortex in the Cat.

The cat's visual cortex contains a number of representations of the visual field by which it is able to perceive features of the external world. Tusa et al. (1981) identified 13 such areas although others may yet be discovered. A representation of one hemisphere of the cat's cortex with a number of visual areas indicated is

presented in Fig 1. Many studies (Rosenquist et al., 1974; Gilbert and Kelly, 1975; Maciewicz, 1975; LeVay and Gilbert, 1976; Hollander and Vanegas, 1977; Geisert, 1980; Raczkowski and Rosenquist, 1983; Bullier et al., 1984a) have demonstrated that areas 17 and 18 are the only areas to receive a projection from the main laminae (A and A1) of the dorsal lateral geniculate nucleus (LGNd). This finding has been used as the basis for regarding areas 17 and 18 alone as constituting the primary visual cortex (Dreher, 1986).

Only Area 17 (the striate cortex) contains a complete representation of an entire hemifield (Tusa et al., 1981). Most extrastriate areas are made up either of areas which contain only the central portion of the visual image, or of areas in which the amount of retinotopic representation devoted to the central percept is considerably greater than that devoted to the peripheral aspect of the image (Dreher, 1986). These regions have been designated areas 18, 19, 20B, 21A, 21B, PMLS (posteromedial lateral suprasylvian), PLLS (posterolateral lateral suprasylvian) VLS (ventral lateral suprasylvian) and DLS (dorsal lateral suprasylvian). In addition there are three regions of the association cortex which specialise in processing visual information from peripheral parts of the visual image. These are areas 20A, AMLS (anteromedial lateral suprasylvian) and ALLS (anterolateral lateral suprasylvian).

Transcortical association pathways from the primary visual cortex terminate in some, but not all, of the association areas (Kawamura, 1973; Zeki, 1978; Van Essen, 1979). In addition, there are several lines of ascending connections which reach the association cortex via transthalamic afferents that synapse in the LP-pulvinar complex (Graybiel, 1972b; Glendenning et al., 1975; Berson and Graybiel, 1978a).

As has been suggested, ablation experiments have shown that the primary visual cortex of the cat may not be necessary for retention and normal learning of pattern and form discrimination tasks (Sprague et al., 1977; Berkley and Sprague, 1979). On the other hand deficits in form perception, but not acuity can result from the lesioning of cortical areas of the middle suprasylvian gyrus including the Clare-Bishop region (Diamond, Fitzpatrick and Sprague, 1985; Berlucchi and Sprague, 1981). This finding adds support to the proposition that areas of the association cortex located in the middle suprasylvian gyrus might be involved with pattern discrimination (Berlucchi et al., 1972; Hara et al., 1974; Cornwell et al., 1976; Sprague et al., 1977; Campbell, 1978).

The cat's visual association cortex also appears to be involved in the transfer of form learning between the two hemispheres. In a split-chiasm experiment bilateral ablation of the middle suprasylvian gyrus resulted in the abolition of inter-hemispheric transfer (Sprague et al., 1981). Where only one hemisphere was lesioned transfer did not occur from the eye on the side of the intact cortex, but did occur in the opposite direction

Area 21a of the Cat's Association Cortex

Area 21 was defined anatomically by Heath and Jones (1971) who set out to demonstrate several organisational aspects of visual centres known to exist in the suprasylvian gyrus. After placing lesions in area 20, a region known to contain a partial topographic representation of the visual field, they noted terminal degeneration in much of the posterior suprasylvian gyrus along the lateral

boundary of area 19, in a position that corresponded closely with Brodman's area 21 in the monkey. They therefore named the region of terminal degeneration, area 21.

Sprague et al. (1977) and Campbell (1978) performed a series of cortical lesion experiments aimed at detecting deficits in visual learning and form discrimination in the cat. Area 21 was amongst the areas studied and they concluded that it was one of several centres thought to be critically involved with pattern discrimination, learning and retention.

The extent and retinotopic organisation of areas 20 and 21 was investigated by Tusa and Palmer (1980) who, on the basis of visual field topography, subdivided both areas into two distinct regions and named them areas 20a and 20b, and 21a and 21b. Their visual field maps indicated that area 21a lies in the caudal part of the middle suprasylvian gyrus and is bounded rostrally by area 7, laterally by areas 21b, VLS and DLS, and medially and caudally by area 19 (see Fig.1.1).

Tusa and Palmer (1980) found that representation of the visual field in area 21a covers only the central 20° and that its transformation to this region is particularly complex. For example, the lower visual field is represented rostrally and the upper field caudally and the two are separated by the area centralis which forms a line across the area. The lower field representation contains a point-to-point, first order transformation of the visual field, whilst the upper field representation is bordered by a representation of the area centralis and the vertical meridian. According to Tusa and Palmer, neurons with the most peripheral receptive fields lie entirely within the upper field rather than forming its border, as is the case with other cortical visual areas in the cat. Despite the fact that some regions near the upper vertical meridian are represented more

FIGURE 1.1

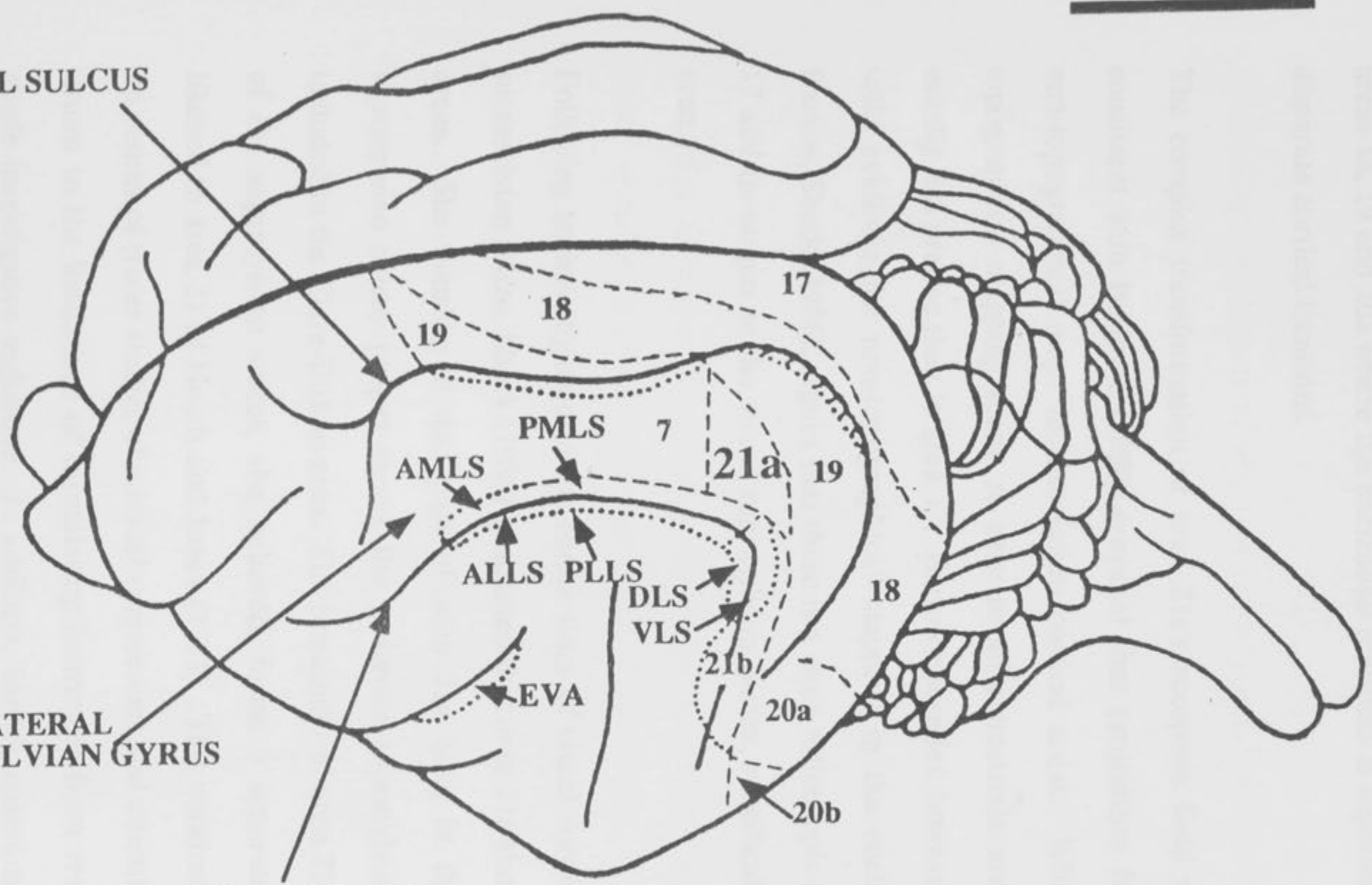
A schematic diagram of the surface of the cat's cortex indicating the location of the main cortical visual areas. Horsley-Clarke co-ordinates are also shown. Area 21a lies in the caudal part of the middle suprasylvian gyrus where it is bounded rostrally by area 7, laterally by areas 21b, VLS and DLS, and medially and caudally by area 19.

1 cm

LATERAL SULCUS

LATERAL SUPRASYLVIAN GYRUS

LATERAL SUPRASYLVIAN SULCUS



--- 0

--- 10

--- 20

+20

+10

0

-10

than once in area 21a and are not contiguous, Tusa and Palmer doubted that the upper field representation resembles a second order transformation as exists in areas 18, 19 and 20a where the horizontal meridian is represented twice in widely disparate cortical locations.

The complex transformation of area 21a's receptive field might appear to be consistent with Barlow's (1980) proposal that projections from area 17 create non-topographical maps in association cortical areas. While some degree of topographical mapping seems to exist in all extrastriate areas, these maps are usually less precise than in area 17. Barlow concedes, however, that there is little solid evidence for non-topographical mapping in the cortex. In contrast to Barlow, Sherk (1986), argues that there is a close retinotopic match between area 17 and its targets in the visual suprasylvian cortex, specifically the Clare-Bishop area.

Following an investigation of the connections of visual cortical areas in the cat's suprasylvian sulcus, Sherk (1986) concluded that area 21a should be split into two areas. She considered that part of area 21a lying in the angle where the suprasylvian sulcus proper meets the posterior suprasylvian sulcus should be included in the Clare-Bishop area. The remainder of area 21a lying on the crown of the suprasylvian sulcus, she believed, forms a separate region which she likened to area 21 of Heath and Jones (1971). This conclusion was based solely on results of tracer studies of cortical connections and attention has already been drawn to the limitations of formulating inferences from results derived from a single investigative technique. In addition, Sherk's criterion for partitioning the cortex into areas, namely the similarity of their afferent connections, is questionable. Whilst she argues that if two regions receive identical connections they are likely to have the same function, electrophysiological evidence suggests

that cortical areas may be multifunctional and therefore, the information an individual area transmits to different regions of the brain may be qualitatively different.

The findings of Grant and Shipp (1991) who combined HRP-WGA axonal tracing experiments with electrophysiological recording from the lateral suprasylvian area and an adjacent region of the ectosylvian gyrus of the cat, were in general agreement with Sherk. They concluded that the medial bank and caudal end of the middle suprasylvian sulcus (MSS) which correspond to areas PMLS, parts of AMLS, PLLS and area 21a, receive a topographically organised projection from the region of area 17 representing the lower visual quadrant. They designated this region, area LS (lateral suprasylvian).

According to Palmer et al. (1978) the medial bank of the MSS contains perhaps three fields of visual representation, each incorporating part of the horizontal meridian (HM). The two caudal fields were grouped to form area PMLS and the rostral field was designated area AMLS. Although Tusa and Palmer (1980) considered that areas PMLS and 21a could be viewed as continuous across a border comprising a representation of the HM, they considered that, on the basis of anatomical findings, the two regions formed separate areas. Support for this view came from Lee, Ho and Dreher (1982) who noted that area 21a, unlike PMLS, receives only a very small proportion of its thalamic afferents from the MIN and parvocellular C layers of the LGNd. Nevertheless, Grant and Shipp claim that the subsequent evidence of Raczkowski and Rosenquist (1983); Symonds and Rosenquist (1984) and Sherk (1986) demonstrate that PMLS and area 21a possess a similar pattern of connectivity and might therefore be considered a single area.

It is questionable whether regions receiving common afferents can unequivocally be grouped to form one area particularly when there are distinct differences in the response characteristics of neurons in each area. Differences in the sharpness of orientation tuning, preferred velocities, directional selectivities and receptive field sizes which exist in areas 21a and PMLS argue strongly that they are separate regions.

Aims and Synopsis of the Research

There have been few anatomical and physiological investigations which concentrated solely on area 21a despite the fact that the area appears to be involved with central vision. Bearing in mind that collective behaviour may not be comprehensible in terms of its constituent parts, the present investigation set-out to explore the role of area 21a in visual perception and to map its pattern of connections with the thalamus and with regions of the cortex. In particular, the research investigated whether there is evidence of functional localisation in area 21a. If it can be demonstrated that the area comprises a separate and complete functional entity, this finding might have important implications for Zeki's doctrine of functional specialisation (Nature, 1978) and for the position of area 21a in the hierarchy of visual processing.

Based on the premise that the function of an area is largely determined by the quality of its inputs, retrograde axonal tracers were used to locate the origin of area 21a's afferent connections. Particular attention was given to the laminar location of cortical inputs to area 21a. In addition, anterograde tracers revealed the terminal destination of axons projecting from area 21a. Results indicated that

area 21a is reciprocally connected with a number of putative visual areas and might be innervated by both transcortical and transthalamic retinal inputs.

CHAPTER 2

Computer-controlled visual stimuli combined with extracellular recording were used to investigate the receptive field location and response characteristics of area 21a neurons. Results confirmed the findings of Tusa and Palmer (1980), Ho et al. (1982), Dreher (1986) and Mizobe et al. (1988) and provided a more detailed description of response features than has hitherto been attempted. Based on the results it was possible to speculate on the functional nature of area 21a and on the location and class of cells from which the area receives its major inputs. A model by which these inputs might be modified in area 21a was proposed.

A further series of experiments was undertaken which involved extracellular recording both before and after controlled cooling of the primary visual cortex. Recordings were made from neurons in areas 21a and 17. The results of these investigations provided supporting evidence of the source of inputs to area 21a and threw light on the excitatory and inhibitory influences which determine the responses of cells in area 21a.

CHAPTER 2

Serial and Parallel Pathways

THALAMIC CONNECTIONS WITH AREA 21A

The concept of serial processing whereby information about the external world flows from the sense organs to the primary cortex and thence to secondary or tertiary cortical areas dates at least from the nineteenth century. The belief that cortical processing depended solely on a transcallosal pathway was so pervasive that much of the association cortex was considered to be "subcortical" (Walter, 1936) or lacking a thalamic input. By the 1950s, however, Hubel and Wiesel (1962, 1963, 1967) also opted for a model of serial processing in which signals pass in serial fashion from one level to a hierarchy of another. Nevertheless, the weight of evolutionary evidence and the results of more sophisticated anatomical techniques suggest strongly against the idea that the visual system of even species as diverse as single-celled amoebae and man consists of a single serial chain of processing centers.

Although the evolution of the neocortex is controversial, it was suggested by a change in the target of the optic tract from the optic tectum to the striatum in mammals (Diamond and Hall, 1967; Henry and Mayhew, 1971). Consequently, with this evolutionary development, the lateral geniculate nucleus (LGN) became a major relay station in the pathway by which visual information reached the cortex. This was especially the case in primates although in many other mammalian orders the superior colliculus (the mammalian optic tectum) continued to be the recipient of

the majority of retinal inputs (Henry and Vidyasagar, 1991) and thus provided an important second visual pathway to the cortex.

INTRODUCTION

A detailed knowledge of the pathways which intervene between the primary and association cortex, as well as the type of information they contain, is essential to

Serial and Parallel Pathways

the operations by which the visual system processes data. Moreover, species differences such as those which exist between monkeys

The concept of serial processing whereby information about the external world flows from the sense organs to the primary cortex and thence to association or secondary cortical areas dates at least from the nineteenth century. The belief that cortical processing depended solely on a transcortical pathway was so pervasive that much of the association cortex was considered to be "athalamic" (Walker, 1938) or lacking a thalamic input. In their early investigations Hubel and Wiesel (1962, 1965, 1967) also opted for a model of visual processing in which signals pass in serial fashion from one level in a hierarchy to another. Nevertheless, the weight of evolutionary evidence and the results of more sophisticated anatomical investigative techniques argue strongly against the idea that the visual system of most species comprises a single serial chain of processing stations.

Thalamic input to the cortex is not the only pathway by which the visual system

Although the evolution of the neocortex in vertebrates was accompanied by a change in the target of the optic tract from the optic tectum to the cortex of the occipital lobe, this evolutionary development did not effect all species equally (Diamond and Hall, 1969; Henry and Vidyasagar, 1991). Concomitant with this evolutionary development the lateral geniculate nucleus (LGNd) became a major relay station on the pathway by which visual information reaches the cortex. This was especially the case in primates although in most other mammalian orders the superior colliculus (the mammalian optic tectum) continued to be the recipient of

the majority of retinal inputs (Henry and Vidyasagar, 1991) and thus provided an important second visual pathway to the cortex.

A detailed knowledge of the pathways which innervate the primary and association cortex, as well as the type of information they conduct, is essential in reaching an understanding of the operations by which the visual system processes data. Moreover, species differences such as those which exist between monkeys and cats are important for the light they throw on departures from a common plan upon which the visual system is based. According to Diamond, et al. (1985) a common plan exists in which there are two pathways carrying information to the neocortex. The older pathway, through the tectum, extends to relay in the LP/pulvinar complex and thence to the visual cortex while the more recent addition passes through the LGNd to the cortex. Early in mammalian history the geniculo-cortical pathway differentiated into the striate branch, concerned with high acuity pattern vision, and an extrastriate branch specialising in motion and coarse pattern vision. The extent of the extrastriate projection involved all the association cortex in more primitive mammals such as the hedgehog (an insectivore) and the opossum (a marsupial). Fewer association areas receive a direct LGNd input in the carnivore and in the primate the number is diminished to the point where almost all the LGNd input takes the path through the striate cortex.

In the case of primates, segregation of thalamo-cortical pathways is achieved, not via extrageniculate routes, but by increased lamination in the striate cortex. The functional significance of additional striate lamination may be related to increased visual specialisation and appears to fit-in with the view that different subdivisions of the extrastriate visual cortex are distinguished by the domination of a particular striate pathway. For example, cells in the monkey middle

temporal area are mainly motion sensitive (Zeki, 1983) while one region within area 18, it has been suggested, is predominantly colour sensitive (Zeki, 1974; Livingstone and Hubel, 1984). The significance of cortical lamination within the cat's visual cortex is discussed further in Chapter 2 of this thesis.

In some species, functional evidence for the existence of a pathway operating in parallel to the primary pathway came from experiments in which regions of the association cortex continued to function even in the absence of inputs from the primary visual cortex. For example, it has been reported that removal of areas 17 and 18 in the cat (the primary visual cortex) produced deficits in acuity but not in form perception, whilst lesions of area 19 and the suprasylvian areas resulted in deficits in form discrimination without affecting visual acuity (Berkley and Sprague, 1979; Berlucchi and Sprague, 1981; Sprague et al., 1981; Kruger et al., 1986, 1988; Kiefer et al., 1989).

More recently, however, DeWeerd et al., (1991) described a series of experiments in which cats were trained in contour orientation discrimination and texture segregation tasks. Their results showed that following bi-lateral ablation of areas 17 and 18 the cats' performance on the tasks was close to chance and there was no recovery despite intensive retraining.

In the case of tree shrews, Diamond et al., (1985) reported that they were able to discriminate depth, colour, patterns and movement in the absence of a striate cortex. Although it should be noted that in this species, extrastriate inputs arise mainly from the pulvinar, not the LGNd (Harting et al., 1973; Luppino et al., 1988). On the other hand Schafer (1888), Kluver (1942), Cowey and Weiskrantz (1963) demonstrated that visual discrimination of monkeys deprived of the striate cortex was severely impaired.

Hierarchical and Parallel Processing in the Cat

The behavioural consequences of these ablation experiments are broadly consistent with the known anatomy. In a comparative assessment of the monkey, the association cortex may be entirely dependent on inputs received from the striate cortex whilst in a number of other species including cats the preservation of some visual functions, without the striate cortex, suggests that the association areas may receive inputs that by-pass the primary visual pathway. In their consideration of the evolution of mammalian visual pathways, Henry and Vidyasagar (1991) point-out that the potential for axons in the optic radiations to bifurcate are considerably less in the primate than in the carnivore with the result that the optic radiations of the primate usually project to a single cortical destination, namely the striate cortex.

Despite evidence for the existence of parallel pathways, the case for a purely parallel model of visual processing, however, cannot be sustained. Evidence from anatomical tracer experiments have demonstrated that parallel pathways have considerable overlap and are more richly connected than originally thought. Consequently, a functional role which is mediated by large numbers of diverging and converging pathways is not easily understood within the framework of a parallel model (Symonds and Rosenquist, 1984). Parallel and serial processing of visual inputs are not mutually exclusive operations and within each stream serial processing must occur as the visual inputs are acted upon at progressively higher levels. Thus both systems can operate simultaneously within the visual system.

Hierarchical and Parallel Processing in the Cat

The dominant extrageniculate thalamic nucleus involved in vision is the lateral

In the cat, the subject of the present study, it has been postulated that there are at least four hierarchical (serial) levels of processing in the visual cortex (Orban, 1984). These are thought to comprise the primary visual cortex (in which Orban includes area 19), the lateral suprasylvian areas (AMLS, ALLS, PMLS, PLLS, DLS and VLS), areas 20 and 21 and the ectosylvian visual area (EVA).

In addition, some thalamic subdivisions have been confirmed by retrograde

Turning to parallel processing, although the path through the LGNd has long been considered a major processing line for visual signals reaching the cortex (Graybiel, 1972), the possibility of a second pathway via the superior colliculus was raised by Altman and Carpenter (1961). Subsequent investigation has produced abundant evidence that parallel pathways innervate the cortex via extrageniculate nuclei (Diamond and Hall, 1969; Graybiel, 1972b; Diamond, 1973, 1976; Glendenning et al., 1975; Berson and Graybiel, 1978a; Rodieck, 1979; Van Essen, 1979; Symonds et al., 1981; Raczkowski and Rosenquist, 1983).

As the

A functional dissection of the geniculo-cortical pathway reveals that the cat's principal cortical afferents arise from the A and A1 layers of the LGNd and terminate solely in cortical areas 17 and 18. Since these areas are the only two retinotopically organised regions to receive direct inputs from the main relay layers of the LGNd through which the principal visual pathway passes, they are considered to constitute the primary visual cortex (Dreher, 1986). In addition, cortical projections arise from the C layers of the LGNd and also from the medial interlaminar nucleus (MIN). Strictly speaking, therefore, there are three ascending LGNd lines of conduction and the potential for three processing pathways. These three, however, are not the only transthalamic routes by which visual inputs reach the cortex.

particularly between the striate cortex and regions of the association cortex

The dominant extrageniculate thalamic nucleus involved in vision is the lateral posterior (LP)-pulvinar complex. This region, which comprises a considerable proportion of the thalamus (Graybiel and Berson, 1981a), has been subdivided into at least three parallel, roughly adjoining zones on the basis of afferent input, architectonic variation and histochemical activity (Rioch, 1929; Graybiel, 1972a, b; Niimi and Kuwahara, 1973; Updyke, 1977; Graybiel and Berson, 1980). In addition, some thalamic subdivisions have been confirmed by retinotopic mapping (Kinston et al., 1969; Mason, 1978; Raczkowski and Rosenquist, 1981). The three zones comprise the pulvinar, the lateral division of the lateral posterior nucleus (LPl) and the medial division of the lateral posterior nucleus (LPm). Although lying side-by-side, there appears to be little interconnection between the zones which are about 1-3mm wide in cross section (Graybiel and Berson, 1981a).

LPm is thought to innervate predominantly areas PM1.2 and PM1.5 as well as projecting less strongly to PM, PMa, PMb and PM1.5 (Dreyer, 1980)

In the cat, the pretectum projects to the pulvinar which is known, therefore, as the *pretectorecipient zone*; the superficial layers of the superior colliculus innervate the LPm which is described as the *tectorecipient zone*; whilst the LPl is the only division to receive an input from the striate cortex and is named, therefore, the *striate- or cortico-recipient zone*. It is noteworthy that, unlike the other divisions of the LP-pulvinar complex which are innervated by sub-thalamic projections, the LPl is almost exclusively innervated from cortical areas particularly the striate cortex (Garey et al., 1968; Graybiel and Nauta, 1971; Graybiel, 1972a; Kawamura et al., 1974; Updyke, 1977; Berson and Graybiel, 1978a; Graybiel and Berson, 1980). The LPl zone probably comprises an important satellite of the geniculostriate system within the extrageniculate thalamus and as a consequence of its involvement in multiple corticothalamo-cortical loops, the region may play a significant role in the exchange of visual information between many cortical areas

particularly between the striate cortex and regions of the association cortex (Berson and Graybiel, 1983).

The orderly division of the LP-pulvinar complex on the basis of incoming visual data is reflected in a similarly ordered plan of efferent projections. This organisational design appears to represent a pattern of thalamo-cortical connectivity in which visual information is conducted along separate paths to a number of areas of the cortex. Each of the three divisions, for instance, is topographically and reciprocally connected with the association cortex (Graybiel, 1972b; Kawamura et al., 1974; Berson and Graybiel, 1978a; Raczkowski and Rosenquist, 1983; Dreher, 1986). The LPm (*tectorecipient zone*) is reported to send major projections to areas PLLS, ALLS, VLS, 20a, 20b, 21b and less robust ones to 19, 21a, DLS, EVA, PMLS and AMLS (Dreher, 1986). The pulvinar (*pretectorecipient zone*) is thought to innervate predominantly areas PMLS and AMLS as well as projecting less strongly to 19, 20a, 21b and PLLS (Dreher, 1986) whilst, it is believed, the LPl (*striate recipient zone*) sends strong projections to areas 18, 19, 20a, 21a, PMLS, AMLS and weaker projections to 17, VLS, 20b, 21b and PLLS (Dreher, 1986).

Area 21a in the Cat

Cat area 21a, the focus of the present study, has been little investigated although it receives multiple cortical and sub-cortical inputs which are known to include projections from the major divisions of the thalamus as well as from the primary visual cortex. This suggests that the area might well hold a unique position in the visual hierarchy placed between other cortical association areas and the primary visual cortex. In this section of my investigation I set out to confirm the afferent supply to area 21a arising from the thalamus and to make an estimate of the

relative strength of the contributions arising from different thalamic divisions. In so doing, my aim was to obtain data which would assist in a comparative assessment of the importance of these pathways in the provision of visual input to area 21a and particularly whether any of them might constitute a secondary visual pathway. These questions are of critical importance in the light of experiments, previously referred to, in which ablation of the cat's primary visual cortex did not result in pattern blindness - a finding which suggested that association areas including perhaps area 21a receive a major transthalamic visual input. In addition, the possibility of cortical-thalamic-cortical loops which might allow for exchanges of information between cortical areas lead to an investigation of whether there existed a topographical "common ground" in the LPI involving axon terminals from striate cells and neurons which project to area 21a. The results of this research was followed by an examination of whether retinotopically corresponding regions of area 21a and the striate cortex receive shared inputs from the LPI.

between Horsley-Clark (HC) coordinates 8.0 and 8.0mm anteroposteriorly, and 9.0 to 13.0mm mediolateral. Coordinates for injection into area 17 were made between HC 10.0 and 12.0mm anteroposterior, and up to 5.0mm lateral from the midline. In each case, a small portion of the dura was then reflected to expose the cortex.

Injection of tracers

In order to investigate the distribution of cells and axon terminals in the thalamus single injections of 4% HRP-WGA (Sigma) in 0.25M Tris-HCl buffer, pH 7.4 were placed in area 21a. As a bidirectional tracer, HRP-WGA labels both cell bodies and terminals. However, in order to provide confirmatory evidence of terminal

MATERIALS AND METHODS

Animal preparation

The tracer experiments were performed on adult cats, weighing between 2.0 and 2.5 kg. Each cat was anaesthetised with an intramuscular injection of ketamine base, 100mg/ml ('Ketamine Injection', Parnell) at an initial dose of 0.4ml, plus 0.3ml of xylazine, 20mg/ml ('Rompun', Bayer). Additional doses of 0.2ml ketamine were administered as necessary during the experiment to maintain anaesthesia. The animal was then placed in a stereotaxic head holder and the skull exposed by a midline incision and retraction of the overlying muscle. Craniotomies for injections into area 21a were made in the left or right hemispheres between Horsley-Clarke (HC) co-ordinates 0.0 and -6.0mm anteroposterior, and 9.0 to 15.0mm mediolateral. Craniotomies for injection into area 17 were made between HC: 0.0 and -7.0mm anteroposterior, and up to 5.0mm lateral from the midline. In each case, a small portion of the dura was then reflected to expose the cortex.

Injection of tracer

In order to investigate the distribution of cells and axon terminals in the thalamus single injections of 4% HRP-WGA (Sigma) in 0.05M Tris-HCl buffer, pH 8.6 were placed in area 21a. As a bi-directional tracer, HRP-WGA labels both cell bodies and terminals. However, in order to provide confirmatory evidence of terminal

locations injections of 4-10% Biocytin (Sigma) in 0.05 μ Tris-HCl buffer, pH 7.4 were used as an anterograde tracer. Injections of both HRP-WGA and Biocytin were placed in area 21a at HC: -2.0 to -3.0mm anteroposterior and 10.0 to 11.0mm mediolateral. The location of the injection sites were determined according to both the topographical co-ordinates of Tusa and Palmer (1980), from appearance of the sulcus pattern which in most instances was visible through the dura and from the results of electrophysiological experiments conducted earlier in our laboratory (Wimborne and Henry, 1992). Injections into area 17 were placed as close as possible to HC: -4.0mm anteroposterior and 2.0mm mediolateral. These injections were within topographical co-ordinates which encompassed the central part of the area's retinotopic representation of the visual hemifield (Tusa et al., 1978). In most cases injection of 0.2-0.5 μ l of tracer was made by means of a 1.0 μ l Hamilton syringe, although occasionally delivery was through a glass micropipette (15-25 μ m tip diameter) by means of a Picospritzer pressure injection device (General Valve Corp.) using 1 to 4x20msec pulses at a pressure of 20psi. Both the Hamilton syringe and the micropipette were tangentially angled at 10⁰ for injections into area 21a, but were vertical for delivery into area 17. Injections were made at depths varying between 0.5-1.5mm below the cortical surface under the control of a micromanipulator, and the delivery apparatus left *in situ* for 20min.

In addition, injections of HRP-WGA were placed in the LPI by means of a 1.0 μ l Hamilton syringe. These injections were made at HC: 7.5mm anteroposterior and 5.0mm mediolateral and the syringe was lowered vertically into the brain to a depth of 13.0mm.

Injections of 0.5 μ l of the fluorescent tracers, Diamadino Yellow Dihydrochloride (DY.2HCl) (DY) and Fast Blue (diamadino compound S 769121) (FB), were

made into areas 21a and 17, respectively, to investigate whether separate populations of neurons in the LPI provided inputs to these cortical regions. The concentration of each tracer was 5-10% and their preparation was based on that described by Bentivoglio et al., (1980) and Keizer et al., (1983). The locations of injections into areas 17 and 21a were the same as those used for injections of HRP-WGA and Biocytin, described above. Delivery was made by means of a Hamilton syringe angled to be normal to the cortical surface and lowered to a depth of 1.0-1.5mm.

Protein sections of Cereb. Cereb. in the case of HRP-WGA and Biocytin experiments, and 25µm thick sections for fluorescent label experiments, were cut on a

Perfusion and fixation HRP-WGA and Biocytin tracers were used, a series comprising every 500 sections was treated with a change in solution to

Animals injected with HRP-WGA or Biocytin were allowed to survive for 48 to 72 hours. In connection with the use of fluorescent tracers, Bullier et al. (1984b) point out that short survival times (these researchers experimented with survival times of 3-8 days) tended to result in fewer labelled cells. On the other hand, they also indicated that a survival time exceeding 8 days often resulted in DY dye leaking into the cytoplasm with some consequent uptake by glial cells. For these reasons a survival time of 7 days was considered appropriate in the present investigation.

After the appropriate survival time the animal was anaesthetised with 2ml sodium pentobarbitone, 60mg/ml ('Nembutal', Abbott Laboratories). Following injection into the heart of 0.3ml heparin (Commonwealth Serum Laboratories), the animal was perfused intracardially with a rapid rinse of normal saline until the superior vena cava had become clear of blood. The perfusion was then continued with 0.7-1.2 litres of fixative over 45min. For HRP-WGA, the fixative consisted of 1% paraformaldehyde/1.25% glutaraldehyde in 0.1M phosphate buffer, pH 7.4.

In the case of Biocytin and the fluorescent tracers, the fixative consisted of 10% neutral-buffered formol saline (NBFS). The brain was then removed from the skull and postfixed for 2hr in NBFS. Brains were subsequently placed in 25% sucrose/phosphate buffer until equilibration.

In addition, a series of sections adjacent to experimentally labelled HRP-WGA regions were prepared for acetylcholinesterase (AChE) histochemistry by the

Preparation of Histological Sections

According to Graybiel and Baran (1988) histochemical identification of the sub-divisions of the lateral posterior-pulvinar

Frozen sections of 50 μ m thickness in the case of HRP-WGA and Biocytin experiments, and 25 μ m thickness for fluorescent label experiments, were cut on a sledge microtome. Where HRP-WGA and Biocytin tracers were used, a series comprising every fifth section was reacted with a chromogen to visualise the labelling, whilst a series of adjacent sections was counterstained with 0.25% neutral red in order to identify cortical layers.

Sections labelled with HRP-WGA were incubated in 3,3',5,5'-tetramethylbenzidine (TMB; Sigma) for 20min, to which was added the substrate H₂O₂ at a final concentration of 0.0075% for 15min. The sections were then rinsed in 0.01M acetate buffer, pH3.3, prior to mounting and dehydration (Mesulam, 1978).

Sections labelled with Biocytin were incubated in a solution of 0.1% HRP-AvidinD (Vector Laboratories) and 1% TritonX-100 in 0.1M phosphate buffer, pH7.4, for 2-4hr (King et al, 1989). After rinsing, the sections were further incubated in 0.05% 3,3'-diaminobenzidine (DAB; Sigma) in 0.05M Tris buffer, pH7.4, for 10min, to which was added H₂O₂ at a final concentration of 0.01% for 20min.

In the case of fluorescent tracer experiments, the mounted sections were examined under fluorescence microscope (viewing condition) without further processing.

In addition, a series of sections adjacent to experimentally labelled HRP-WGA sections were prepared for acetylcholinesterase (AChE) histochemistry by the Karnovsky and Roots (1964) method. According to Graybiel and Berson (1980) histochemical identification of the sub-divisions of the lateral posterior-pulvinar complex and related thalamic nuclei, including the LPI (striate recipient zone) may be achieved using this method.

RESULTS

HRP-WGA Labelling in the Thalamus

Thalamic labelling was observed in eight cats in which area 21a was injected with HRP-WGA. Figure 2.1 presents a photomicrograph of a typical HRP-WGA injection. In this example the injection has spread through both supragranular and infragranular laminae. As a result of the injection, labelling was observed in several zones of the ipsilateral thalamus. The presence of filled cell bodies and axon terminals indicated the efficacy of the tracer for both anterograde and retrograde transport. A schematic diagram prepared with the aid of a drawing tube mounted to a microscope summarises this labelling (Fig. 2.2). This figure illustrates five coronal sections through the thalamus commencing at HC: +5.0mm and extending to HC: +9.0mm, with each section displaced 1.0mm from its neighbour. Extensive labelling was apparent and the photomicrographs presented in Figs. 2.3 A, B and C record how the labelled cells were distributed in the LPI-pulvinar complex and the LGNd.

(1) LPI

Labelling of cells and terminals was most evident in a location that appeared to correspond to the LPI. When viewed in cross-section, an elongated band of dense labelling extended across the thalamus in a dorsal-ventral plane towards the lateral ventricle. The labelling is indicated by arrows in Figs. 2.3A, 2.3B and 2.3C which are a sample of three coronal sections through the thalamus. Labelling was present in sections ranging from HC: +5.0mm to +8.0mm anteroposterior and reached its highest density at HC: +7.0mm where it measured about 2mm x

FIGURE 2.1

A photomicrograph of a typical HRP-WGA injection site in area 21a. In this example the injection was made via a Hamilton syringe angled at 10° and inserted to a depth sufficient to deliver the tracer into all cortical laminae. The scale bar = 1mm.

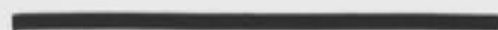
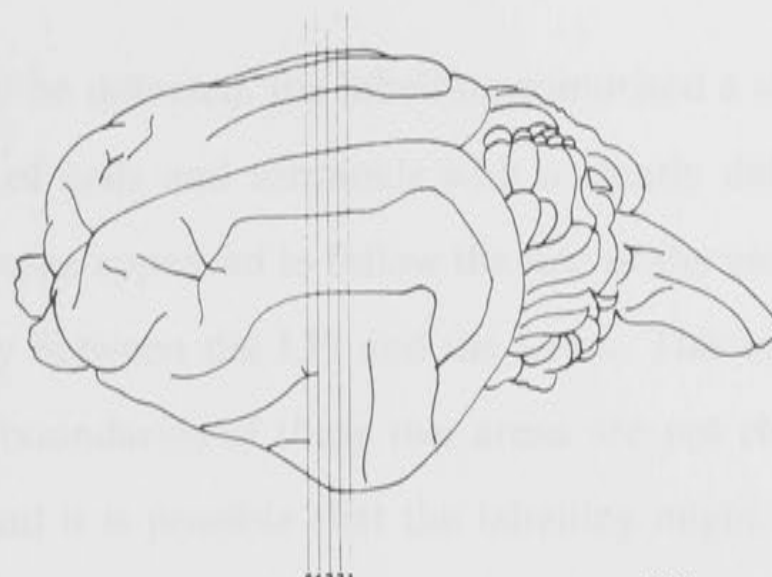


FIGURE 2.2

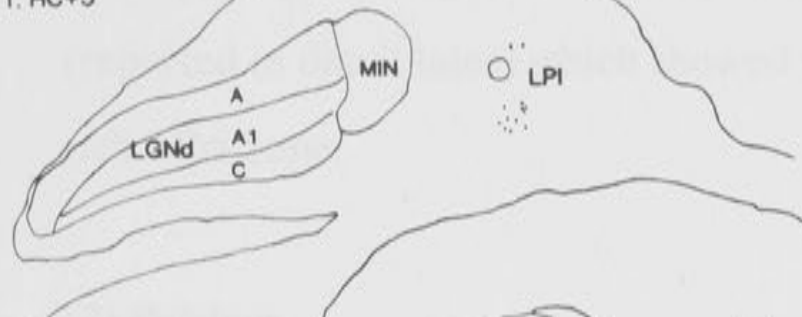
Drawings of five coronal sections through the diencephalon each 1mm apart which show areas of labelling that resulted from injections of HRP-WGA into area 21a. Labelling, which is indicated by stippling, was apparent in the pulvinar, LPI, LGNd and LGNv. The locations of the illustrated sections which extend from HC +5.00 to +9.00mm AP are shown in the inset.



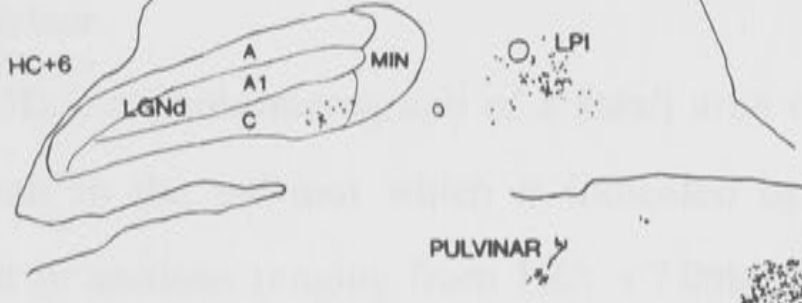
54321

5mm

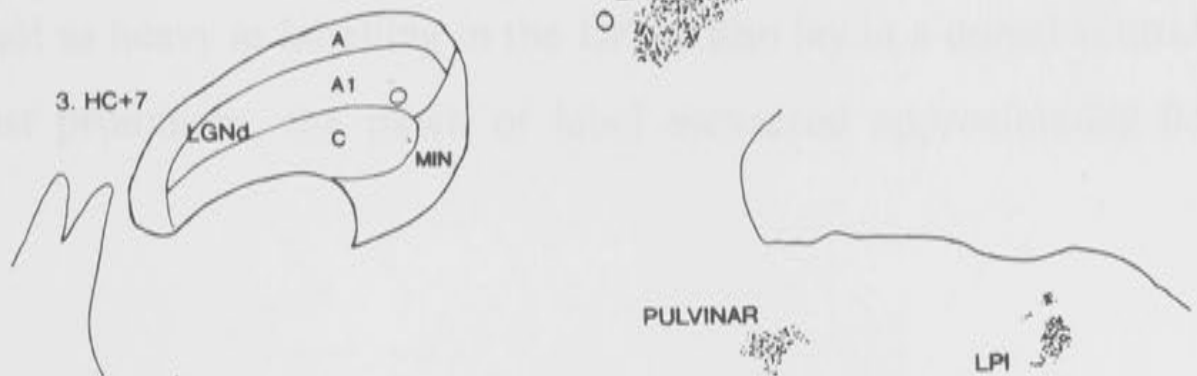
1. HC+5



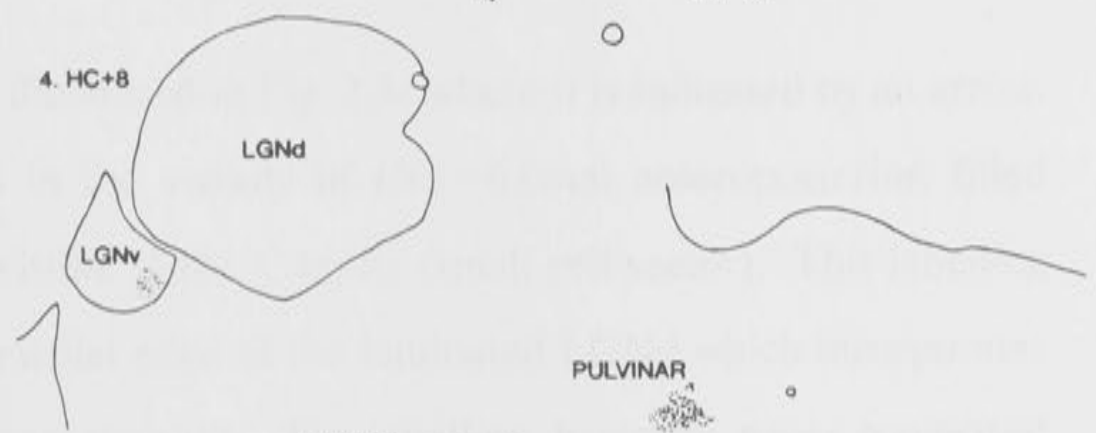
2. HC+6



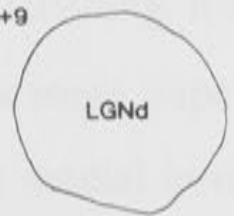
3. HC+7



4. HC+8



5. HC+9



1mm

1mm.. In all sections where it could be detected, the labelling comprised a single cluster which often formed a slab of cells and terminals with a clearly defined edge. In many sections the medial edge appeared to follow the line of the vertical meridian which forms the boundary between the LPl and the LPm. This is best seen in Figs. 2.3A and 2.3C. The boundaries of these two areas are not clearly defined in Nissl stained material and it is possible that the labelling might have overlapped both regions. This possibility is discounted, however, by results (reported in detail later) which showed that labelling did not occur in the ACh.E-rich LPm zone.

(2) Pulvinar

Fig. 2.3B is a photomicrograph of a small area of labelled cell bodies and axon terminals in the pulvinar which is indicated by an arrow. This labelling was present in sections ranging from HC: +7.0mm to +9.0mm anteroposterior and although not as heavy as labelling in the LPl, it also lay in a dorsal-ventral plane. At its most prominent, the patch of label measured approximately 0.5mm x 0.5mm.

(3) LGNd

Labelling in the LGNd is illustrated in Fig. 2.3c where it is indicated by an arrow. In several serial sections in the vicinity of HC:+6.0mm anteroposterior, filled cells and terminals were visible in the C layers (small cell sector). This labelling was located towards the medial edge of the laminated LGNd which incorporates a representation of the area centralis. The labelling, however, never comprised more than a few cells and terminals and in some experiments it could not be identified with certainty. No labelling in the medial intralaminar nucleus (MIN) was apparent.

FIGURE 2.3

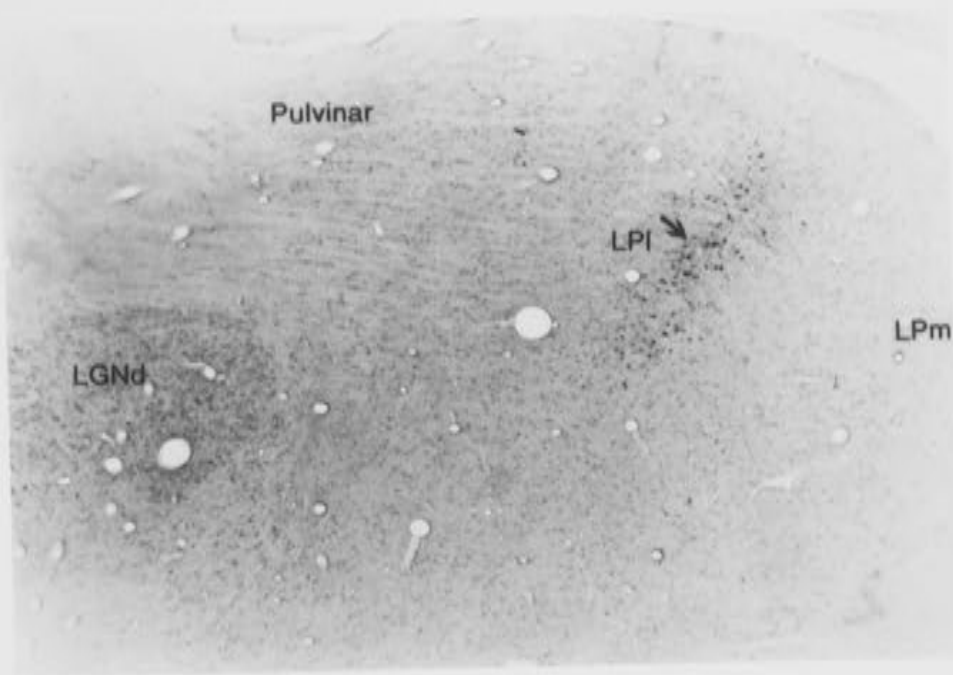
Photomicrographs of three coronal sections through the diencephalon depicting areas of labelled cells and axon terminals which resulted from HRP-WGA injections into area 21a. In each photomicrograph the positions of the pulvinar, LGNd, LPl and LPm are indicated. The scale bar = 1mm.

Fig. 2.3(A) shows dense labelling in the LPl, indicated by an arrow. The labelling has a slab-like appearance lying in a dorsal-ventral plane. The medial edge of the labelled patch appeared to follow the line of the vertical meridian which forms the boundary between the LPl and LPm.

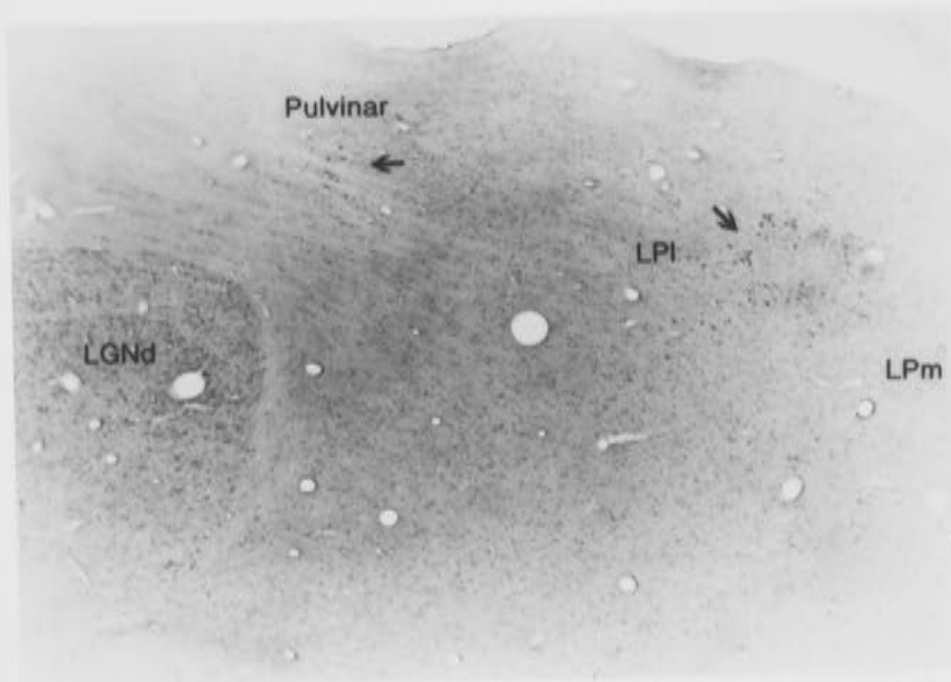
Fig. 2.3(B) depicts some sparsely filled cells and axon terminals in the pulvinar and some light labelling in the LPl, indicated by arrows.

Fig. 2.3(C) shows the very sparse labelling of cells and boutons found in the C layers of the LGNd and is indicated by an arrow. This labelling occurred in the vicinity of HC +6.0mm AP and was present in only a few coronal sections. Some labelling, as shown by another arrow, was still evident in the LPl.

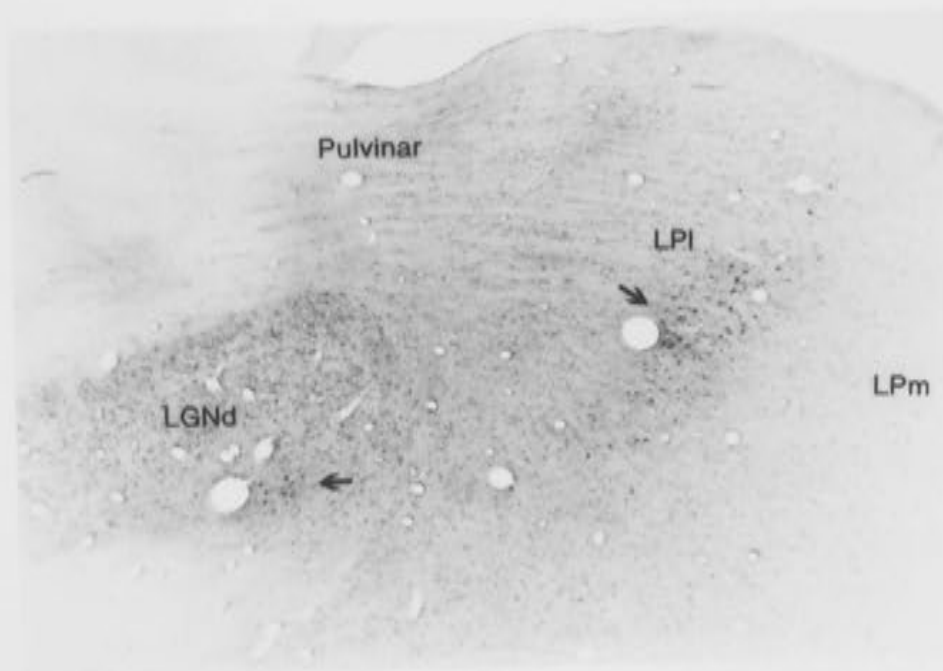
(A)



(B)



(C)



(4) *LGNv*

Axon terminals were densely labelled in the ventral lateral geniculate nucleus (*LGNv*) at HC: 8.0mm anteroposterior but there was no evidence that cell bodies in the *LGNv* had taken-up the tracer. The labelling was located in the dorsal half of the *LGNv*.

The existence of robust reciprocal connections between the LPI zone and area 21a as indicated by the dense labelling of cells and terminals, lead to an experiment on two additional animals where HRP-WGA was injected into the LPI. The aim of this experiment was to determine the laminar location of cells and terminals in area 21a which are connected with the LPI. It was difficult to limit the injection to the LPI although it was clear from the photomicrograph (Fig. 2.4A) of one of the injection sites that label was centred on the LPI. Fig. 2.4B is a coronal section through area 21a showing that the injection resulted in heavily labelled cells throughout lamina 6. Although not obvious from this photomicrograph, lamina 4 was characterised by labelled axon terminals. The labelled cells in lamina 6 and terminals in lamina 4 were visible in a number of other cortical areas. For example, the labelled cells appeared as a continuous band through areas 17, 18, 19 and was also evident in areas 7, 20a, 20b, 21b, PMLS (posterior medial lateral suprasylvian), AMLS (anterior medial lateral suprasylvian) and SVA (splenial visual area). Dreher (1986) does not report on projections from the LPI to area 7 and SVA. Raczkowski and Rosenquist (1983), however, suggest that areas 7 and SVA receive connections from the pulvinar. There is the possibility, therefore, that the injection may have spread into the pulvinar.

FIGURE 2.4

Fig. 2.4(A) is a darkfield photomicrograph of a coronal section through the diencephalon showing an HRP-WGA injection site centred on the LPI. The injection site, which may have spread into the pulvinar, is visible as the large white patch adjacent to the dorsal-medial edge of the thalamus. The scale bar = 1mm.

Fig. 2.4(B) is a darkfield photomicrograph of a coronal section through the cortex showing the labelling of cells in area 21a which resulted from the injection described in Fig. 2.4(A). The labelling of cells was restricted to lamina 6 and, although not apparent at this magnification, axon terminals in lamina 4 were also labelled. The scale bar = 1mm.

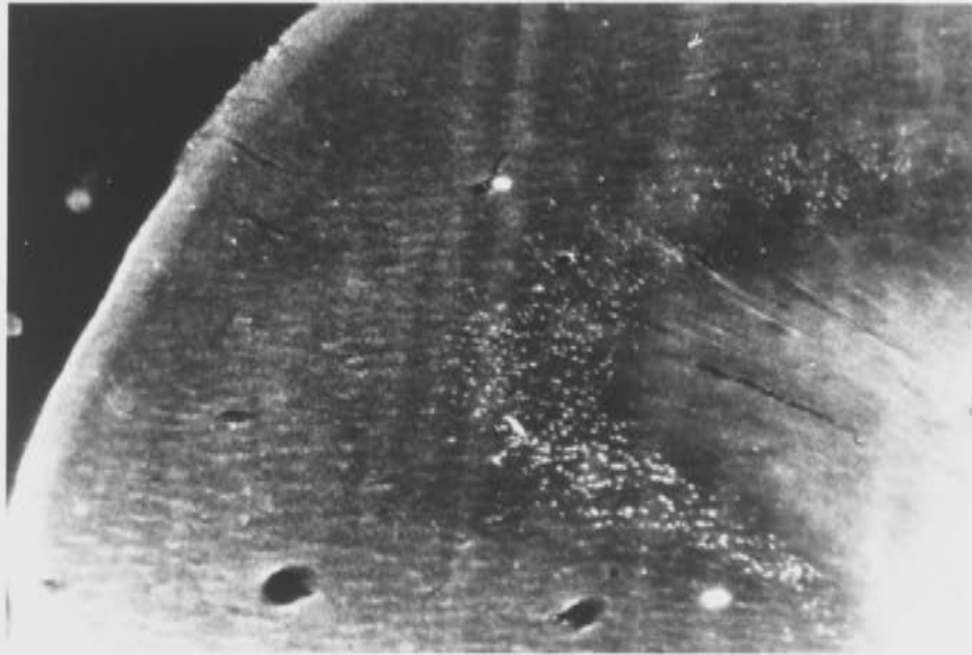
DORSAL

(A)



LATERAL

(B)



MEDIAL

VENTRAL

LPI Projection to Area 21a and Distribution of AChE

The tracer experiments with HRP-WGA showed that the cells projecting from the LPI to area 21a were located close to a zone of the LPI which, on the basis of previous tracing experiments (Wimborne, 1989), was known to connect strongly with area 17. Although in Nissl stained sections, there are no visible divisions between them, the three main zones of the LP-pulvinar complex can be identified histochemically by virtue of their content of acetylcholinesterase (AChE) (Berson and Graybiel, 1983). Of the three divisions, the LPI or *striate recipient zone* stains comparatively weakly for AChE (Graybiel and Berson, 1980; Berson and Graybiel, 1983) and stands out as a light band when viewed with dark field illumination.

In order to investigate the relative locations of cells projecting to areas 17 and 21a, a detailed examination was undertaken of the degree of correspondence between labelling in the LPI, which resulted from injection of HRP-WGA in area 21a, and its apparent counterpart in adjacent AChE stained sections. Once the existence of HRP-WGA labelled cells in the LPI had been established, therefore, alternate sections were processed for the presence of AChE. Results of this experiment are shown in Fig. 2.5. A deposit of HRP-WGA injected into area 21a resulted in the labelling pattern in the thalamus illustrated in Fig. 2.5a. The AChE staining pattern in a serially adjacent section is shown in Fig. 2.5b. Figures 2.5c and 2.5d illustrate a second pair of adjacent sections which have been subjected to the same experimental procedure. Matching blood vessels which provide useful fiducial markers that assist in comparisons of the pairs of sections are indicated by asterisks. Arrows in Figs. 2.5b and 2.5d indicate the position of the boundary between regions which are heavily stained (to the right) and lightly

FIGURE 2.5

Four photomicrographs of coronal sections through the diencephalon showing the distribution of acetylcholinesterase (AChE.) and HRP-WGA labelled cells projecting from the LPI to area 21a. Arrows indicate the position of the border between the LPI and LPm as demonstrated by the distribution of AChE. In each pair of photographs, (a) (b) and (c) (d), matching blood vessels which provide useful fiducial markers that assist in comparisons of pairs of sections are marked by asterisks. The scale bar = 1mm.

Fig. 2.5(a) shows labelled cells in the LPI which resulted from an injection of HRP-WGA into area 21a. The filled cells form a slab-like band lying in a dorsal-ventral plane.

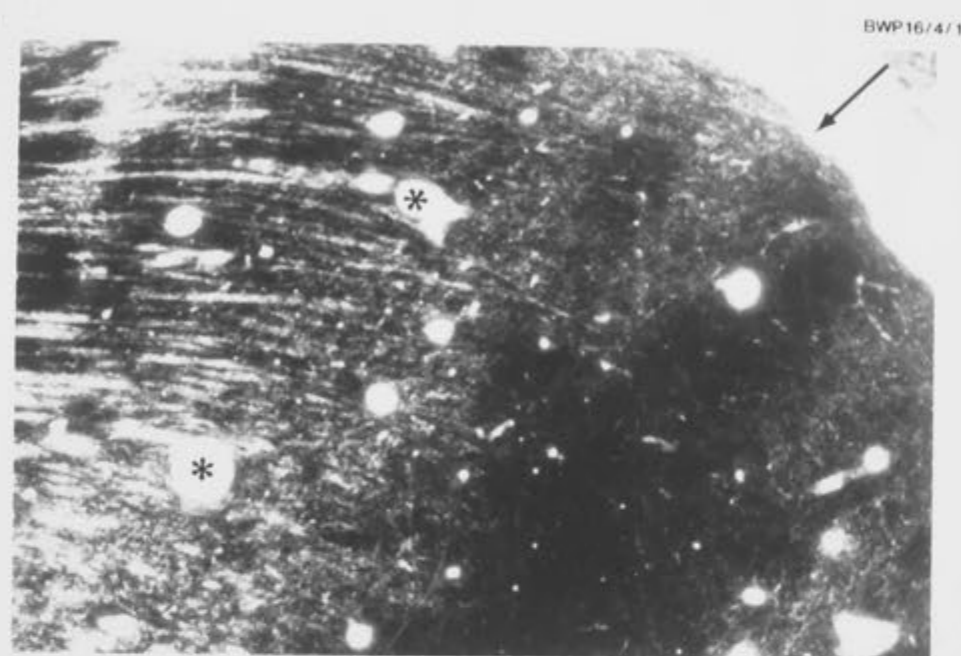
Fig. 2.5(b) is an adjacent section which has been processed for the presence of AChE. The region which stains comparatively weakly for AChE. represents the striate recipient zone of the LP-pulvinar complex. It is apparent that HRP-WGA labelled cells shown in Fig. 2.5(a) are located in this zone.

Figs. 2.5(c) and 2.5(d) represent a second pair of adjacent sections which have been subjected to the same experimental procedure described for Figs. 2.5(a) and 2.5(b).

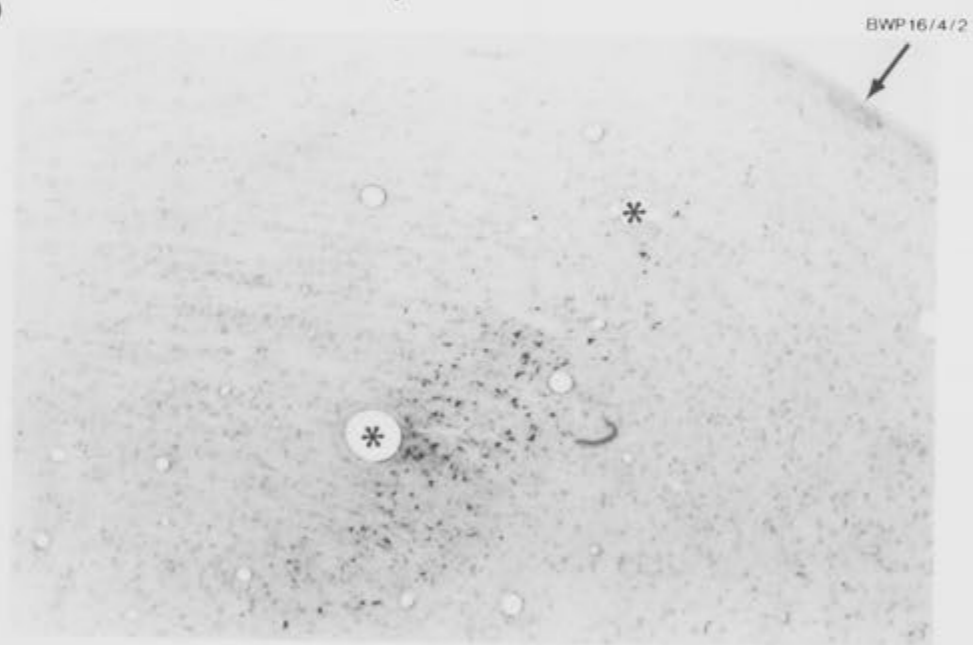
(a)



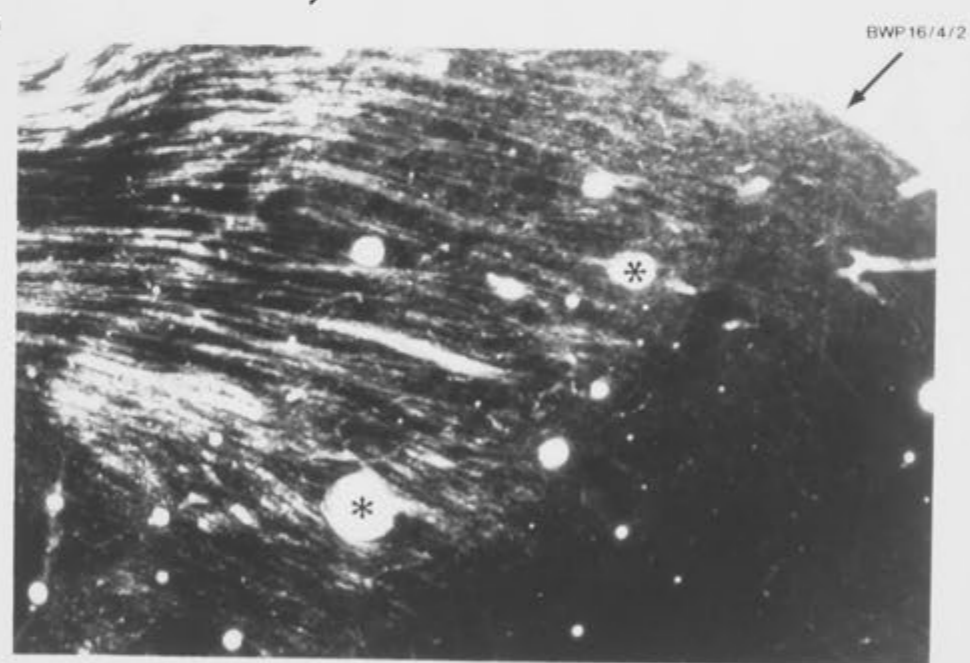
(b)



(c)



(d)



stained for AChE. The position of this border in the HRP-WGA labelled sections (Figs.2.5a and 2.5c) is indicated by corresponding arrows.

The series in Fig. 2.5 demonstrate the close correspondence between the cluster of HRP-WGA labelling in the LPI and the area which stained weakly for AChE (i.e. the *striate recipient zone*). The slab-like appearance of the HRP-WGA labelling is apparent in Fig. 2.5a and its medial edge borders the AChE-rich zone in Fig.2.5b which designates the LPm (see Graybiel and Berson, 1980; Berson and Graybiel, 1983). The labelled cells showing the connection of the LPI with the striate cortex and with area 21a were located in the *striate recipient zone*. To check if individual cells in the LPI projected to both areas, an additional experiment was undertaken, in which simultaneous, separate injections of fluorescent tracers were made into areas 17 and 21a and the LPI was later examined for the presence of double-labelled cells.

Double Fluorescent Labelling in the LPI

The aim of this experiment was to demonstrate, with a higher degree of accuracy, the relative positions in the LPI of cells projecting to area 21a and to the striate cortex, respectively and, as mentioned above, to search for cells common to each pathway. Two cats were used in an experiment in which Diamadino yellow (DY) was injected into area 21a and Fast blue (FB) injected into that region of the striate cortex thought to contain a central retinotopic representation of the visual hemifield (Tusa et al., 1978). Both fluorescent dyes are effective axonal tracers. DY is transported over long distances in the cat and produces a yellow fluorescence of the neuronal nucleus at 360 nm excitation wavelength (Keizer et al., 1983). FB is also transported over long distances and, at the same excitation

wavelength, labels the cytoplasm of the cell body with a blue fluorescence (Bentivoglio et al., 1980).

These experiments resulted in the labelling of two populations of cells in the LPI. DY labelled cells were the more profuse and formed an extensive patch which spread across the LPI medial-laterally. The cells were first visible at about HC: +7.0mm anteroposterior and continued serially until HC: +5.0 mm. Cells labelled with FB were less numerous and were present in only a few sections at HC: +6.0mm. However, they were not topographically displaced from the DY labelled cells. Fig. 2.6 presents photomicrographs of this labelling magnified X1250. An example of DY labelled cell bodies is presented in Fig. 2.6A. The two nuclei in the centre are typical of the dense DY labelling in the LPI. Fig. 2.6B is a section containing separate cells labelled with each dye. In this illustration a nucleus is labelled with DY and part of the cytoplasm of another cells is labelled with FB. A further example of cells labelled with FB is shown in Fig. 2.6C. In this illustration one labelled cell is especially prominent, but a close examination of the photomicrograph reveals evidence of more extensive labelling. There was no evidence, however, of double labelled cells.

The relative paucity of FB labelled cells in the LPI could not be attributed to any failure in uptake at the area 17 injection site since densely FB labelled cells were apparent in the LGNd and MIN in sections ranging between HC: +5.0 and +7.0mm anteroposterior. Furthermore, the retinotopic locus of the injection site in area 17 was confirmed in the LGNd by FB labelling close to the medial edge of the nucleus which contains a representation of the central portion of the visual field. Photomicrographs of fluorescent labelling in the LGNd and MIN are also presented in Fig. 2.6. These were magnified X1250. In Fig. 2.6D FB labelled cells in the LGNd are illustrated. This labelling was confined to the A layers. FB

FIGURE 2.6

The relative positions in the thalamus of projections from areas 17 and 21a, respectively, were demonstrated with the use of fluorescent tracers. Diamadino yellow (DY) was injected into area 21a and fast blue (FB) into area 17. The resulting labelling in the thalamus is described in Figs 2.6(A) to 2.6(F). The scale bar = 50 μ m

Fig. 2.6(A) shows two nuclei in the LPI filled with DY which resulted from an injection into area 21a.

Fig. 2.6(B) depicts two cells in the LPI. The nucleus of one is labelled with DY and the cytoplasm of another is labelled with FB. This labelling resulted from injections of DY and FB into areas 21a and 17, respectively.

Fig. 2.6(C) shows a FB labelled cell in the LPI which resulted from retrograde transport from area 17.

Figs. 2.6(D) and (E) present two examples of FB labelled cells in the the A layers of the LGNd, close to the medial edge of the nucleus which contains a representation of the central portion of the visual field. The location of this labelling demonstrated that the injection of FB into area 17 was at a position corresponding to the central retinotopic representation.

Fig. 2.6(F) shows a single DY labelled nucleus in the C layer of the LGNd which resulted from the DY injection into area 21a.

labeling in the MDV is presented in Fig. 2. The majority of the MDV are located in the subepithelial layer C of the CTM. The majority of the MDV are located in the subepithelial layer C.

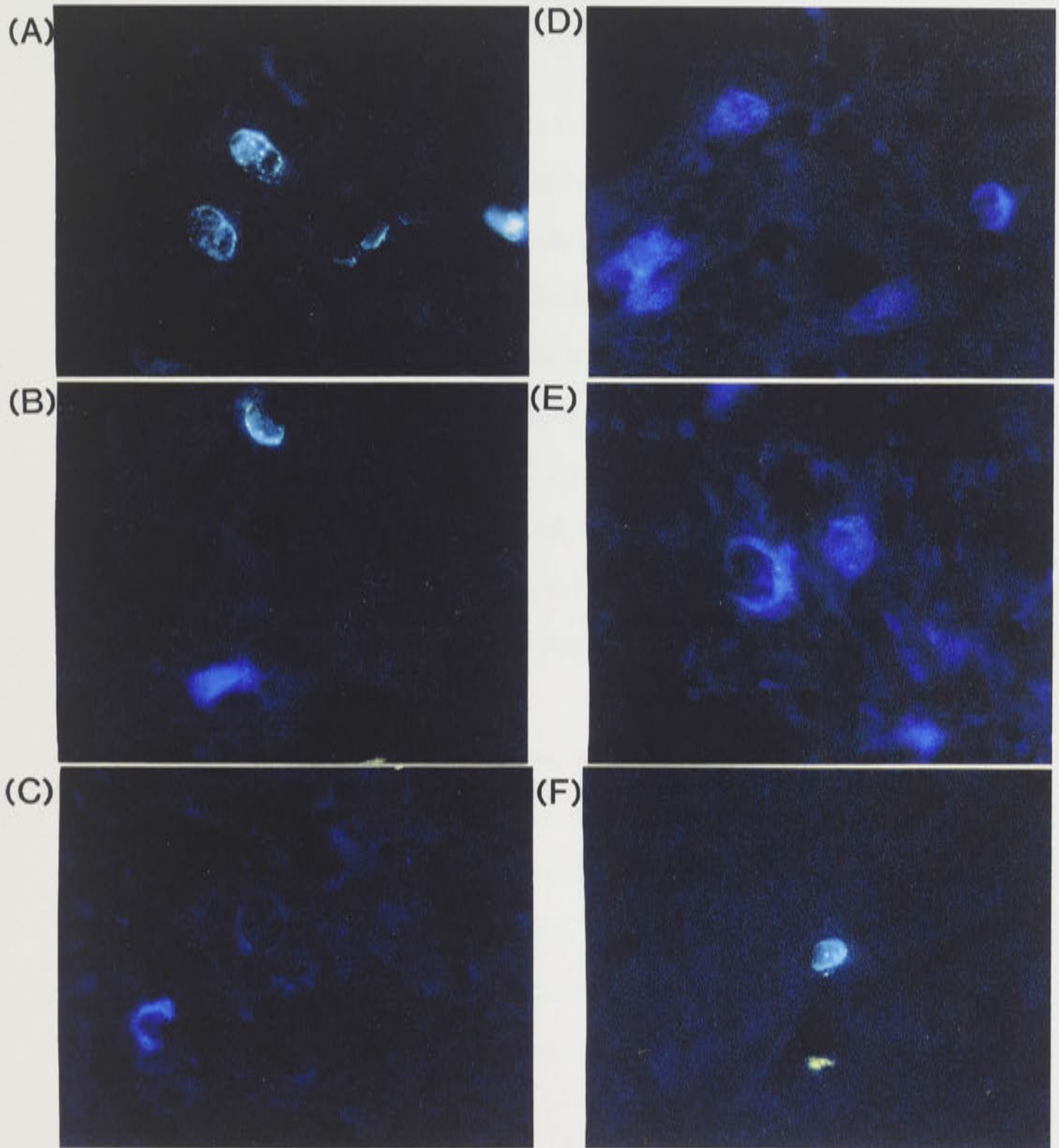


Fig. 2. MDV in the subepithelial layer C of the CTM. The majority of the MDV are located in the subepithelial layer C. The majority of the MDV are located in the subepithelial layer C.

labelling in the MIN is presented in Fig. 2.6E. The single cell in Fig. 2.6F is a DY labelled nucleus in layer C of the LGNd which resulted from the DY injection into area 21a.

Biocytin Labelling in the Thalamus

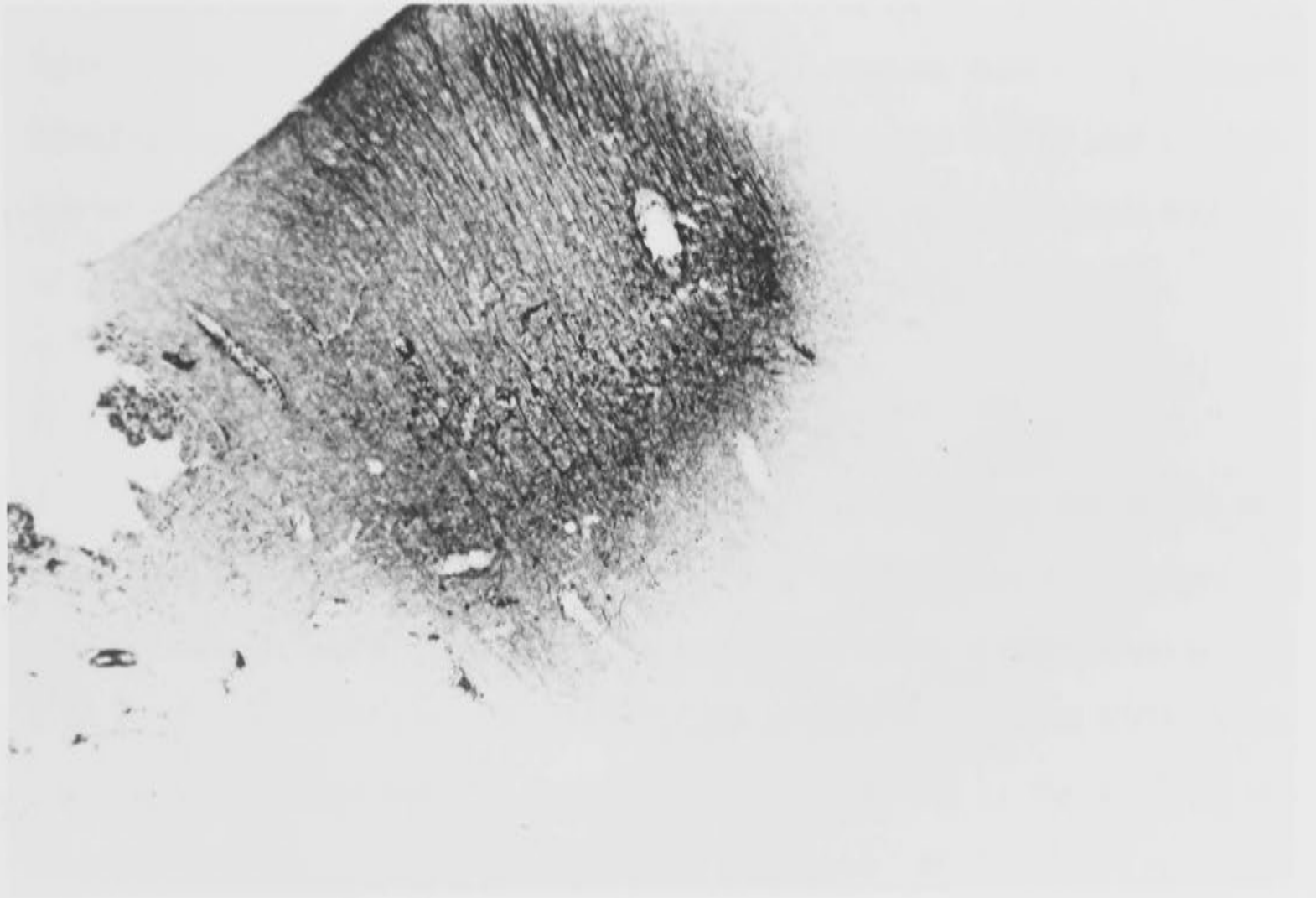
Labelling of terminals in the thalamus resulted from injections of area 21a with HRP-WGA and indicated that this tracer operates bi-directionally. Nevertheless, injection of area 21a with an anterograde tracer was deemed advisable in order to confirm this result and to provide more clearly identifiable labelling of terminals. In the early experiments electrophoresis was used to deliver the tracer, Phaseolus vulgaris leucoagglutinin (Pha-L). Procedure followed that set-out by Henry et al., (1990). However, despite the apparent uptake of the tracer at the site of injection in some cases, there was no evidence of labelled terminals or fibres outside area 21a. This indicated that axonal transport over distance had not occurred. As a consequence, it was decided to experiment with an alternative anterograde tracer.

Experiments using the tracer, Biocytin, were then undertaken. Unlike HRP-WGA, Biocytin appears, over distance, to be mainly transported anterogradely and very few retrogradely filled cells could be distinguished outside the cortex. Four cats were used in this investigation. A typical Biocytin injection site in area 21a illustrated in Fig. 2.7. shows Biocytin-filled processes in all laminae extending from the pial surface to the white matter.

In terms of labelled regions of the thalamus, the outcome of Biocytin experiments was in keeping with that which resulted from the anterograde transport of HRP-WGA. Labelled axon terminals and fibres were present in the LPI, pulvinar,

FIGURE 2.7

Photomicrograph of a coronal section through the cortex depicting a typical injection of Biocytin in area 21a. Axons filled with Biocytin extended from the pial surface to the white matter. The scale bar = 1mm.



LGNd and LGNv. The density of labelling in these structures varied between light to moderate and resolution of the fine axon terminals usually required relatively high power. Figure 2.8 presents a drawing tube illustration of labelling present in a sample of eight coronal sections through the thalamus. The insets, B1 and H1 display more highly magnified labelling in the LPI and LGNd (C layers), respectively (see scale bars). In this illustration, most of the thalamic labelling was confined to fibres in the LPI, pulvinar and LGNd and gives the appearance of more extensive labelling than resulted from anterograde transport of HRP-WGA. A more detailed description of this labelling follows.

(1) LPI

The densest label occurred in the LPI where it spread across the region in a dorsal-ventral plane. Figure 2.9 presents three photomicrographs of representative labelling in the LPI taken at different levels of magnification. Fig. 2.9A illustrates part of a heavily labelled patch of the LPI magnified X125. In this example an extensive mesh of fine fibres can be seen but at the relatively low magnification, the terminals are difficult to distinguish. In Fig. 2.9B a portion of the LPI illustrated in the previous figure has been magnified X500. In this case both fibres and terminals are more readily distinguishable although the processes are very fine. Several types of terminals can be identified including clusters and single boutons on stalks but the most commonly occurring terminals were single boutons which appeared to protrude from the surface of the axon. Fig. 2.9C is a photomicrograph (X1250) of a section of the axon which extends across the centre of Fig. 2.9B. Although it appears as one of the more prominent fibres in the previous figure, its diameter is estimated at less than $0.5\mu\text{m}$ and there is little indication of varicosities occurring along it. The diameter of the boutons is estimated to be about $1\mu\text{m}$.

FIGURE 2.8

Drawing tube illustrations of Biocytin labelling in eight coronal sections through the thalamus which resulted from an injection of the tracer into area 21a. In this example the labelled regions comprised the LPI, pulvinar and LGNd. The insets, B1 and H1, show magnified labelling in the LPI and LGNd (C layers) respectively.

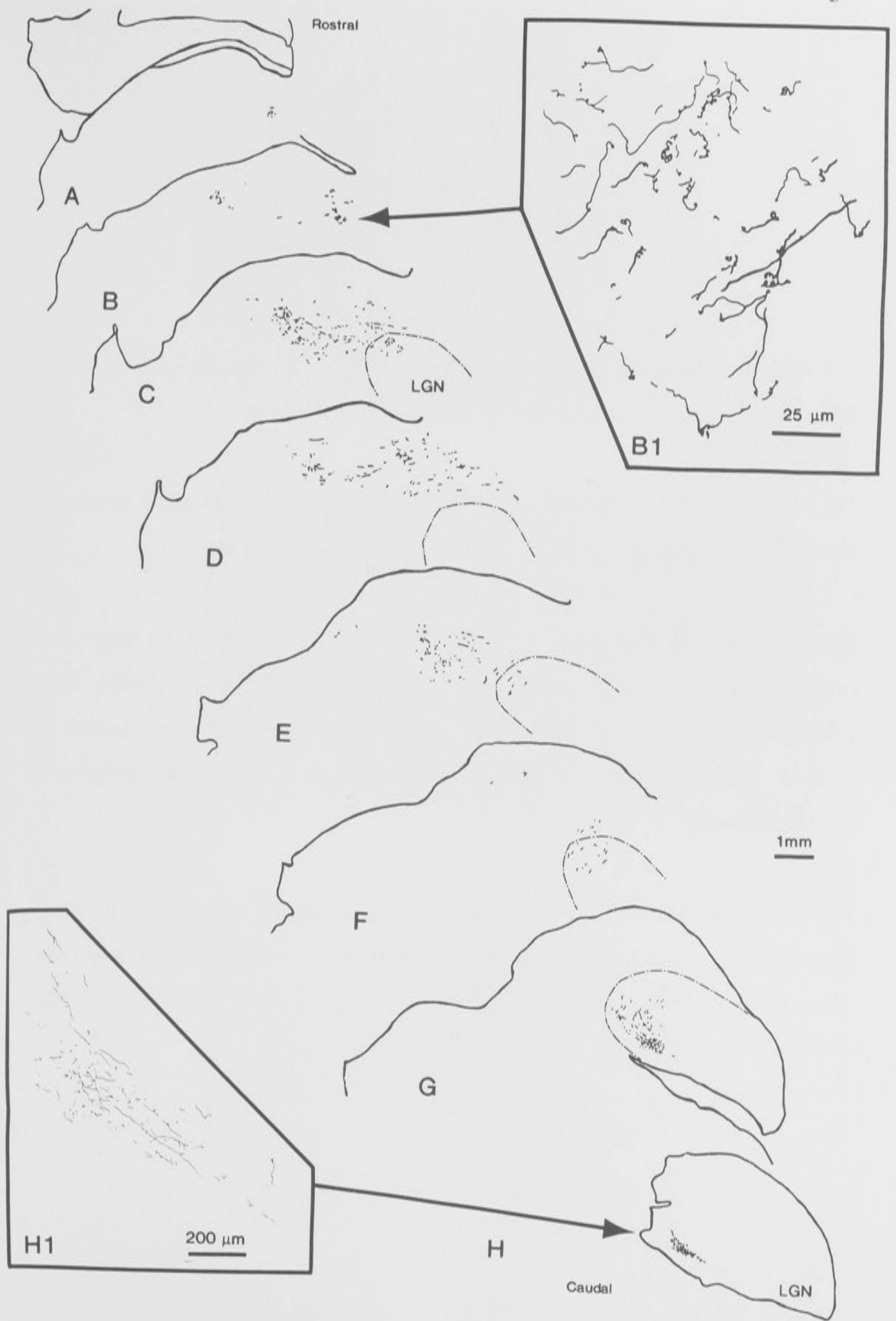


FIGURE 2.9

Photomicrographs, taken at three levels of magnification, of Biocytin labelling in the LPI which resulted from an injection of the tracer into area 21a.

Fig. 2.9(A) is a densely labelled section of the LPI magnified X125 which illustrates an extensive network of filled fibres.

Fig. 2.9(B) is a section of the previous figure magnified X500. This shows the presence of fine processes and several types of axon terminals which included single boutons on the surface of the axon or protruding on stalks, and small clusters of boutons. Individual axons often seem to terminate in fine threads which synapse on a number of cells.

Fig. 2.9(C) is a section of the axon which extended across the centre of the previous figure magnified X1250. The diameter of the fibre is estimated to be less than $0.5\mu\text{m}$ and that of the boutons to be about $1\mu\text{m}$. Axon terminals appear to lie on the surface of the fibre or to protrude from short stalks.

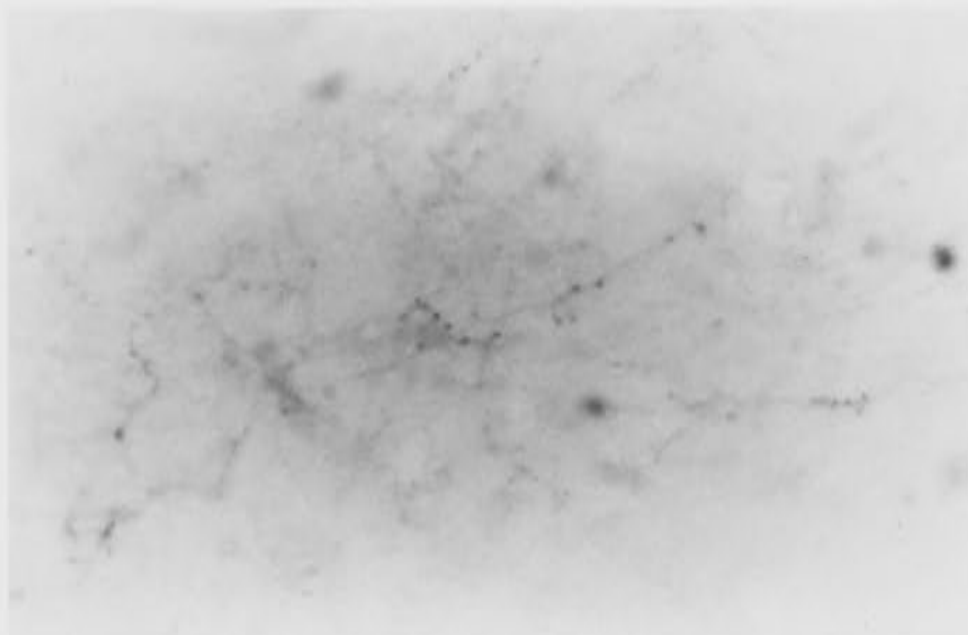
LPI

(A) Mag. x 125



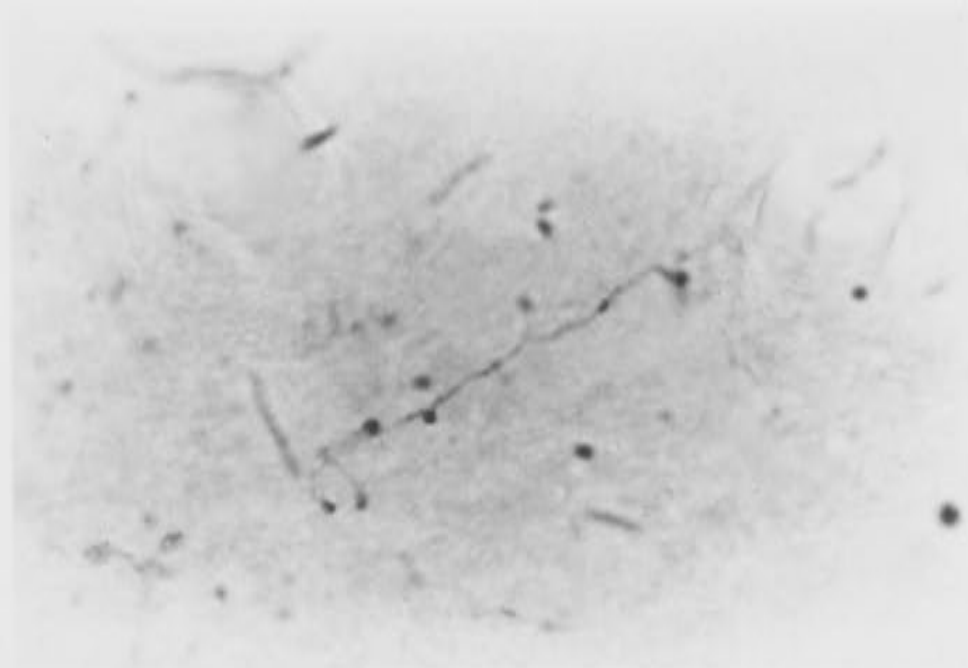
100 μ m

(B) Mag. x 500



25 μ m

(C) Mag. x 1250



10 μ m

Fibres extending across the LPI could often be traced for several hundred microns. They seldom ended in terminal tufts on dendrites and it appeared that each axon terminated in a single fine thread that made synaptic contact with a number of cells. Typical examples of Biocytin labelled fibres and terminals are shown at a magnification X125 in Fig. 2.10. Fig. 2.10A presents a photomicrograph of a section of the LPI showing these characteristically long fibres whilst a drawing tube illustration of the identical section is shown in Fig. 2.10B. Although most fibres in the LPI were fine, a common feature which is especially apparent in the photomicrograph was that others were of considerably larger diameter. Often these fibres could be traced for relatively long distances. This pattern of connectivity suggests a possible loss of retinotopic integrity since individual neurons from area 21a, with restricted visual field representation, make contact with cells spread across the nucleus in the LPI.

(2) Pulvinar

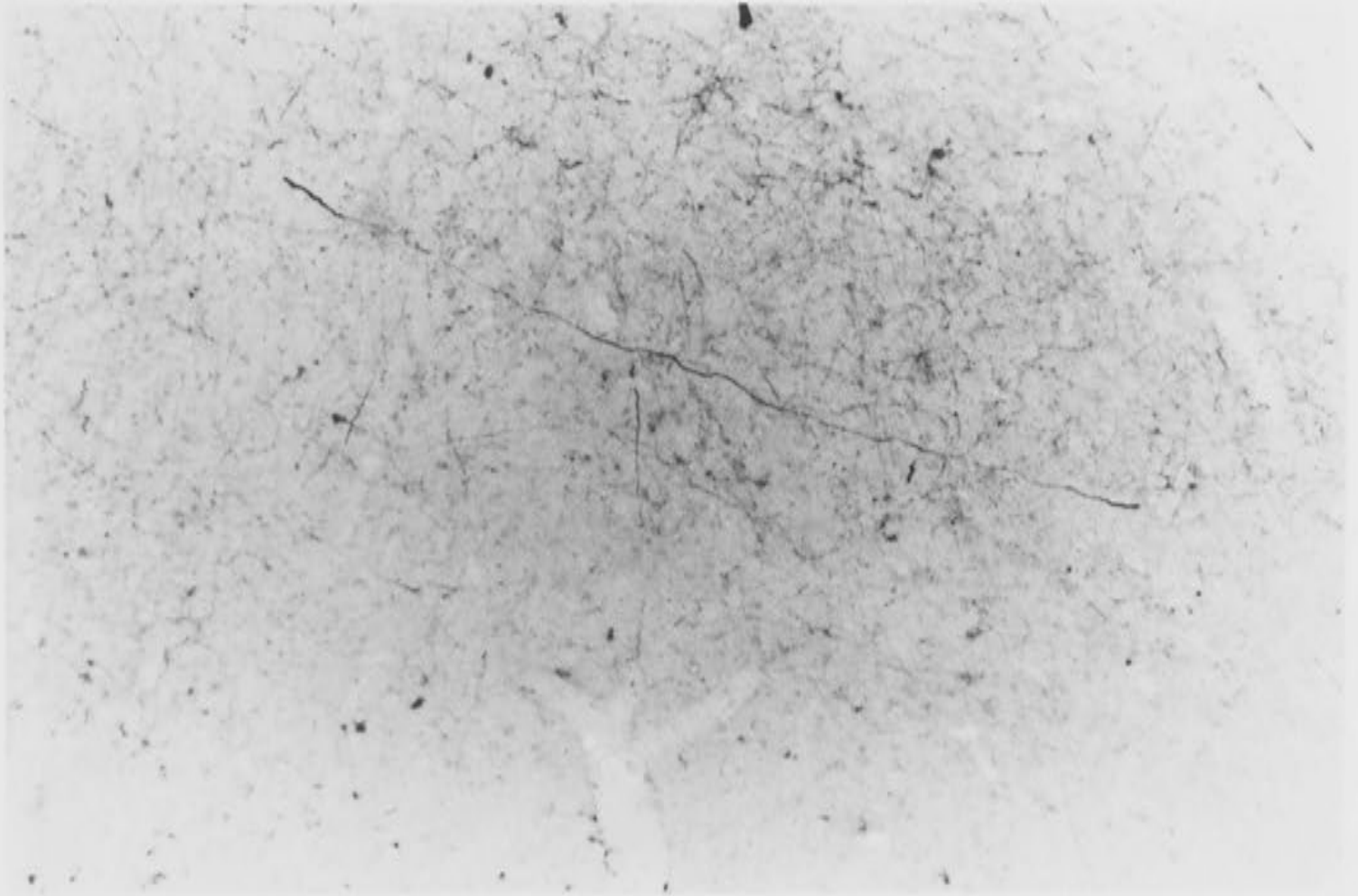
Prominent labelling occurred in the pulvinar where it appeared as a circular dense patch of fibres and terminals located medial-dorsal to the LGNd. Figure 2.11 presents photomicrographs of this labelling taken at a range of magnifications. In Fig. 2.11A the full extent of labelling in the pulvinar is shown. At a low magnification (X125) the labelling appears as a dense mesh of fibres.. In Fig. 2.11B the central portion of the pulvinar magnified (X500) revealed the presence of long axons. The most common type of axon terminal appeared to be single boutons on stalks which often protruded from the axon. Nevertheless, unlike the pattern of labelling in the LPI, some terminal arborisation also occurred. At higher magnification (X1250) in Fig. 2.11C the diameter of the fibres could be measured at about $0.5\mu\text{m}$ whilst that of the terminals appears to vary between 0.5 and $1.0\mu\text{m}$.

FIGURE 2.10

Fig. 2.10(A) is a photomicrograph of a section of the LPI densely labelled with Biocytin. Note the presence of long fibres which could often be traced across the LPI for several hundred μm . Many of these fibres were relatively thick, indicative perhaps, of heavy myelination.

Fig. 2.10(B) is a drawing tube illustration of the same section of the LPI showing several of the characteristically long fibres.

(A)



(B)



50 μm

BC1 SLIDE A2

FIGURE 2.11

Photomicrographs, taken at three levels of magnification, of labelling in the pulvinar which resulted from an injection of Biocytin into area 21a.

Fig. 2.11(A) is a small dense patch of labelling taken at a magnification X125 showing a fine network of fibres.

Fig. 2.11(B) is the central section of the previous figure magnified X500. This depicts a pattern of long fibres of varying diameter and numerous axon terminals many of which appeared to be located on short stalks protruding from axons.

Fig. 2.11(C) illustrates part of the previous figure magnified X1250 to show a section of a long axon and several axon terminals. The diameter of the axons is estimated at around $0.5\mu\text{m}$ and that of the terminals at $0.5\text{-}1.0\mu\text{m}$.

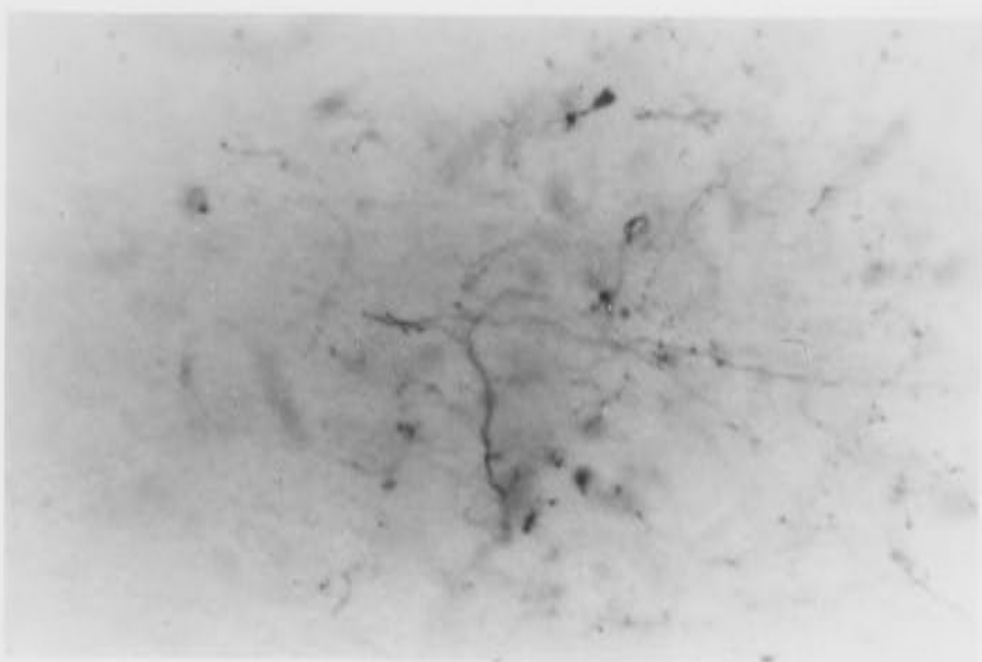
PULVINAR

(A) Mag. x 125



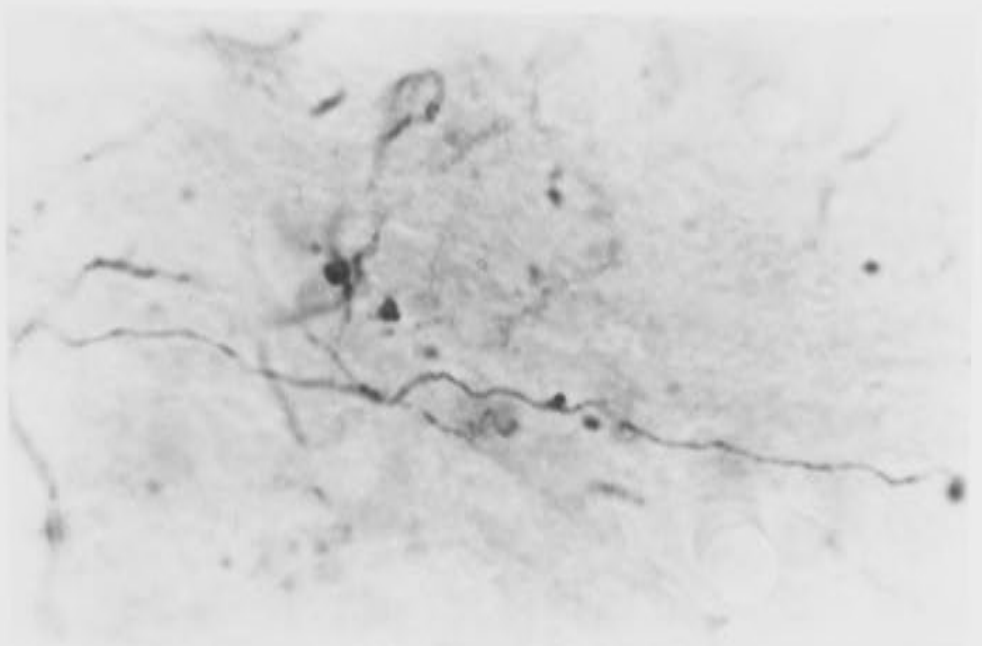
100 μm

(B) Mag. x 500



25 μm

(C) Mag. x 1250



10 μm

(3) LGNd

Labelling of terminals and fibres in the LGNd was confined to the C layers and was evident in only half a dozen serial sections at about HC: +5.0mm AP. Labelling was located towards the medial edge of the nucleus but it was not extensive and in some experiments its presence could not be established with certainty. Photomicrographs of sections containing the most prominent labelling found in the LGNd are presented in Fig. 2.12 at three magnifications. The full extent of labelling is apparent in the coronal section (X125) in Fig. 2.12A in which a network of fine fibres is visible. A portion of this labelling (X500) is presented in Fig. 2.12B. In the top left hand corner can be seen the ventral edge of the vessel which appears in Fig. 2.12A. Numerous terminals and fibres are visible. As was the case in the LPl, the individual axons appeared to terminate in a fine thread which synapsed on a number of cells. However, the axons were not as long as those in the LPl. Fig. 2.12C of the same section at higher magnification (X1250) shows a segment of a fine fibre with an estimated diameter of $0.5\mu\text{m}$ and several axon terminals with diameters of up to $1\mu\text{m}$.

(4) LGNv

Figure 2.13 presents photomicrographs of labelling in the LGNv taken at three magnifications (X125, X500 and X1250). The labelling was evident in a range of serial sections at around HC: +7.0mm. Filled fibres and terminals were prominent throughout this structure and are visible in all three illustrations. In Fig 2.13A the full extent of labelled terminals and fibres is apparent in this section (X125). A single fibre stood alone in the right half of this field. It was heavily labelled and appeared to terminate in the centre of the picture in a dense arborisation of fine fibres about $1/4\text{mm}$ in diameter. The diameter of the thick fibre is estimated to be between $5 - 7\mu\text{m}$. An enlargement (X500) of the central

FIGURE 2.12

Photomicrographs, taken at three levels of magnification, of labelling in the LGNd which resulted from an injection of Biocytin into area 21a.

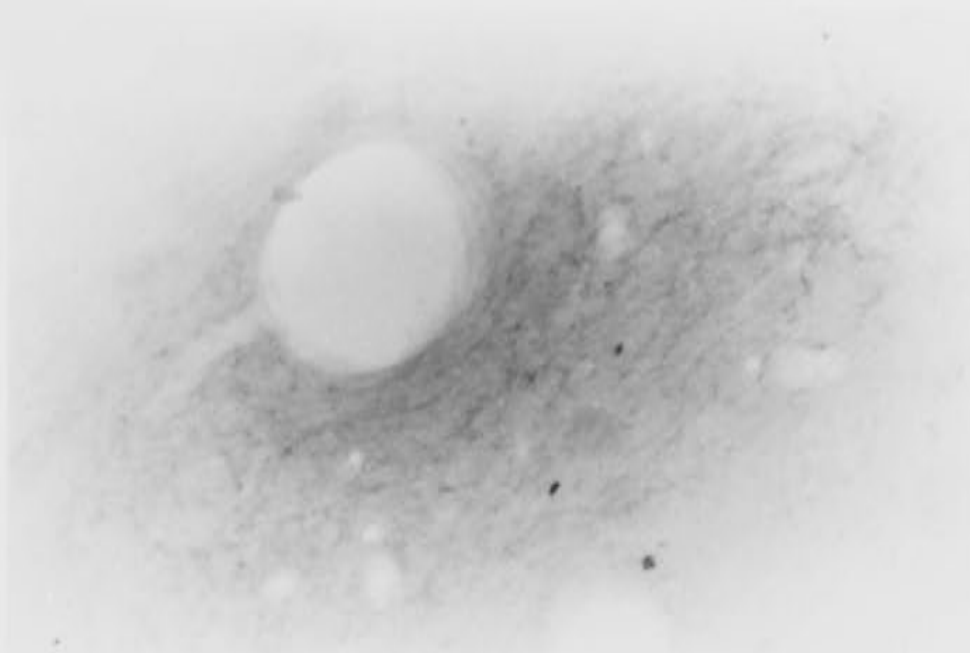
Fig. 2.12(A) is the only example of labelled fibres and axon terminals in the LGNd. It comprised a small patch that was confined to the C layers and was never visible in more than a few sections.

Fig. 2.12(B) shows a portion of the labelling magnified X500 which illustrates a network of fine fibres and terminals. The fibres are comparatively short but they often terminated in fine threads which synapsed on a number of cells.

Fig. 2.12(C) depicts a segment of a fine fibre and several axon terminals magnified X1250. The diameter of the axons is estimated to be about $0.5\mu\text{m}$ and that of the terminals at a little under $1\mu\text{m}$.

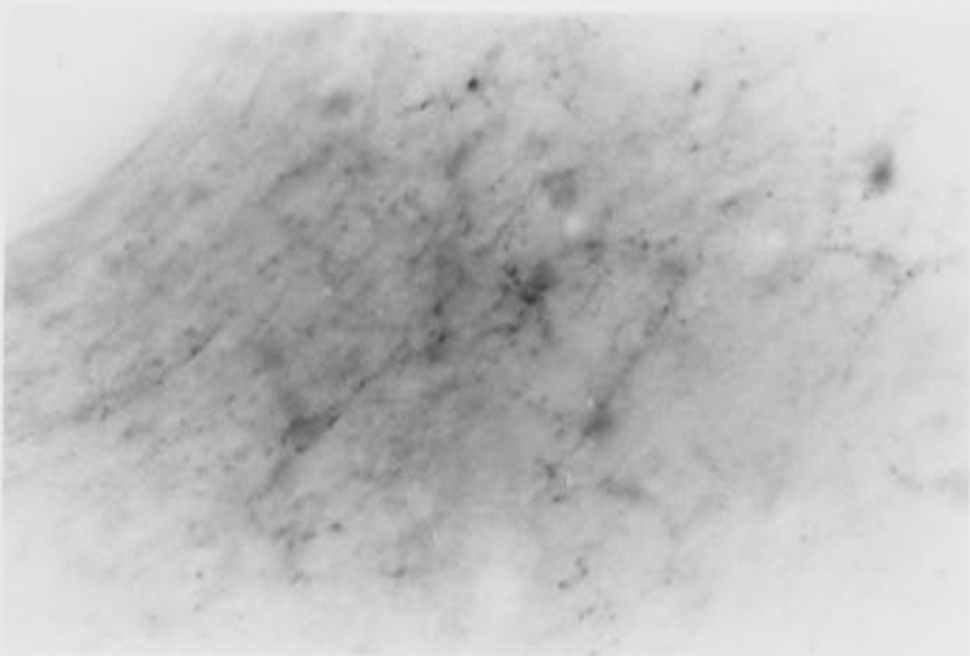
LGNd

(A) Mag. x 125



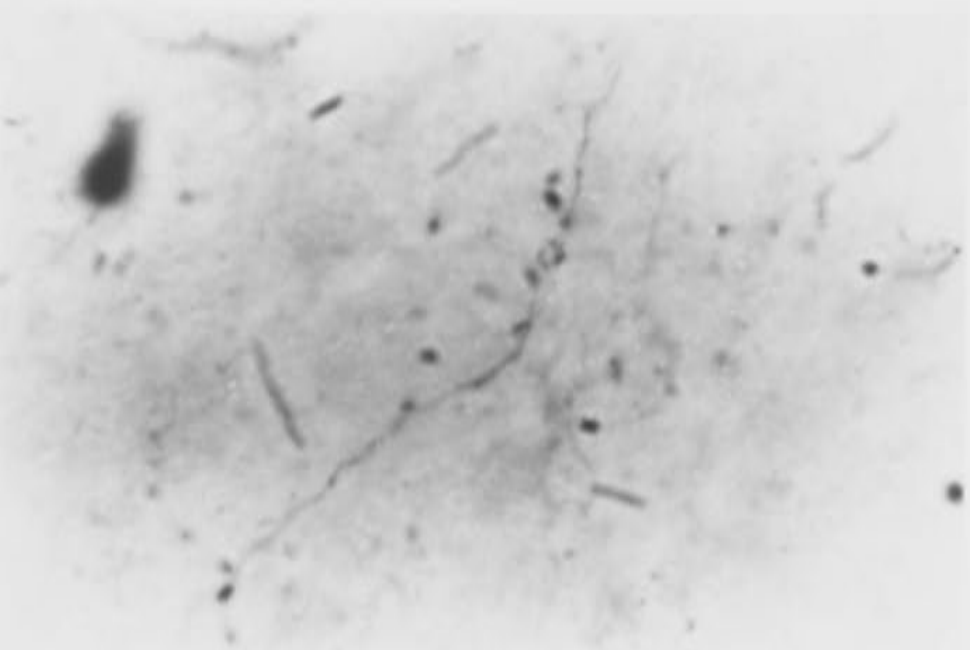
100 μ m

(B) Mag. x 500



25 μ m

(C) Mag. x 1250



10 μ m

portion of this fibre short of the labelled arbor is presented in Fig. 2.13B which shows a number of heavy branches which quickly break down to form a mass of fine terminal twigs. At higher magnification (X1250) as shown in Fig. 2.13C frequent varicosities characterised the axons, the diameters of which are estimated to be 1 - 3 μ m. The diameter of the terminals is about 1 μ m.

FIGURE 2.13

Photomicrographs taken at three levels of magnification of labelling in the LGNv which resulted from an injection of Biocytin into area 21a.

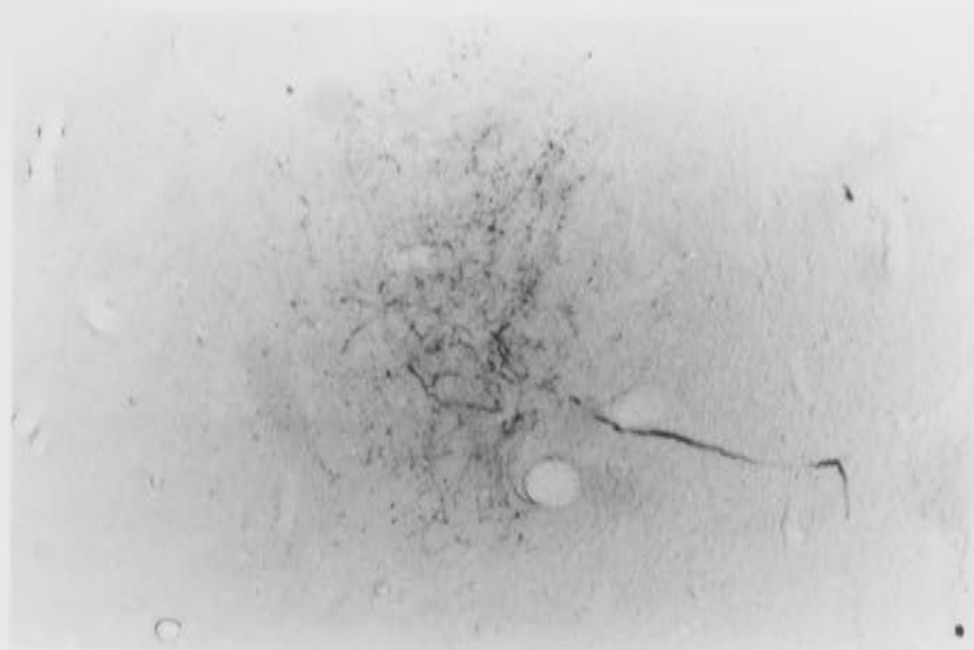
Fig. 2.13(A) shows densely filled fibres and axon terminals magnified X125. A single heavily labelled fibre in the right half of the field appears to terminate in the centre of the illustration in a dense arborisation of fine fibres. The diameter of the single fibre is estimated at $5-7\mu\text{m}$ and that of the region of arborisation is about $1/4\text{mm}$.

Fig. 2.13(B) is an enlargement X500 of the central portion of the previous figure depicting the segment of the thick axon short of the labelled arbor and many heavy branches which break down to form a mass of terminal twigs.

Fig. 2.13(C) is a small section of the thick axon magnified X1250 showing varicosities which were frequently present along its length. Several axon terminals are also illustrated. The diameter of the axon is about $1-3\mu\text{m}$ and that of the terminals around $1\mu\text{m}$.

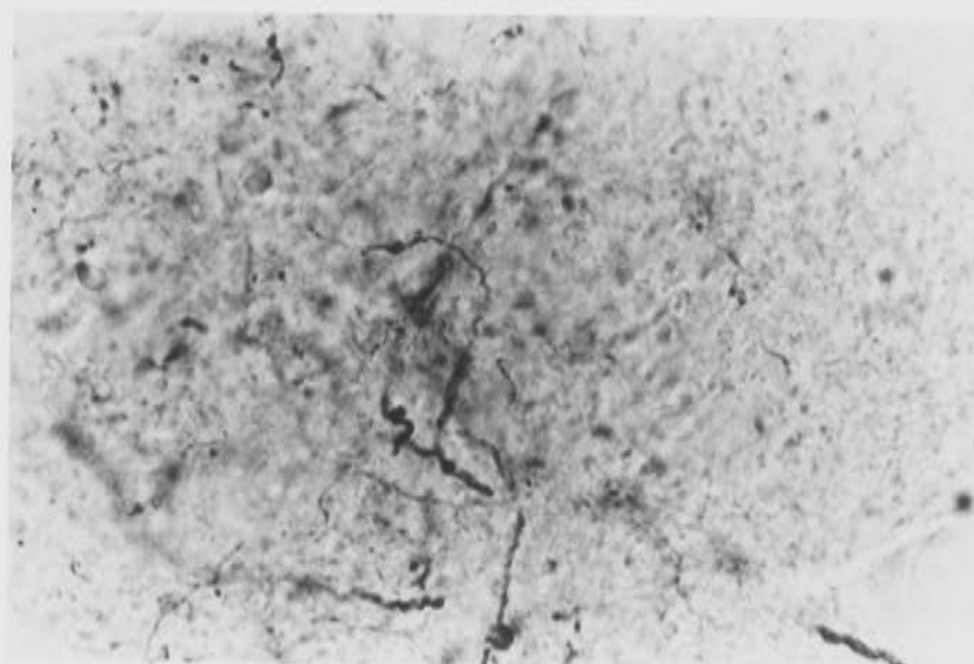
LGNv

(A) Mag. x 125



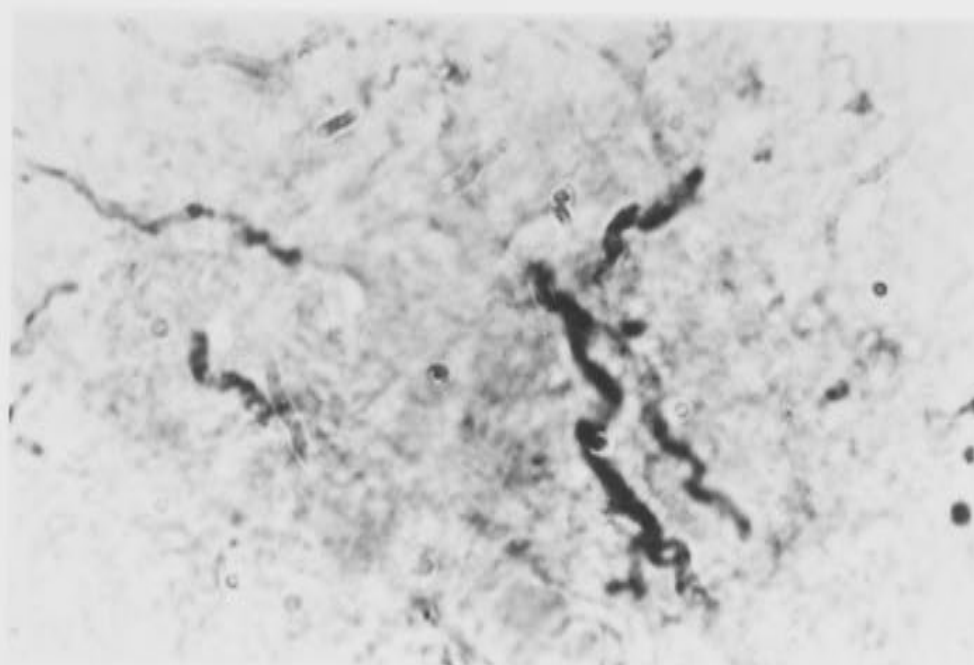
100 μ m

(B) Mag. x 500



25 μ m

(C) Mag. x 1250



10 μ m

DISCUSSION

Whether the numerous representations of the visual field, which have been mapped in the association cortex, are derived from the primary visual cortex or whether they comprise true parallel representations of retinal inputs transmitted through the thalamus are key issues. In the present study, as was mentioned in the Introduction, the proposition that, in the cat, visual association areas receive their input exclusively from the primary visual cortex has been challenged by experiments in which the removal of areas 17 and 18 did not apparently result in pattern blindness. Although this result points to the existence of a parallel visual pathway which innervates non-primary cortical areas, it cannot be presumed that area 21a, as part of the association cortex, is the recipient of such a pathway. If it can be demonstrated that area 21a's input is derived solely from the primary visual cortex, in distinction to other association areas that have additional non-striate inputs, it would follow that area 21a has a different role in the sequence of visual processing.

Although there is strong evidence that area 21a receives its principal input via the pathway through the LGNd and the primary visual cortex (see Chapters 2 and 5) there remains the equally relevant issue as to the nature of other pathways which innervate the area through the thalamus. Creutzfeldt (1985) has drawn attention to the question of whether area 21 carries a path that runs parallel to the various outputs from area 17 or whether the cells of area 21 are more influenced by the inputs transmitted to the cortex through the thalamus. The evidence from the present investigation indicates that area 21a is innervated from at least three zones of the thalamus - the LGNd, pulvinar and LPI. Whilst the LPI receives its input from regions of the cortex, signals to the LGNd originate directly from the

retina whilst those supplying the pulvinar, although they also come from the retina, do so largely by way of the pretectum. Hence, the ascending projections from the LGNd and the pulvinar to area 21a have the potential to operate in parallel to the primary visual pathway through areas 17 and 18. Of special relevance to the functional significance of these connections is their density as revealed in the tracer experiments.

Relative Strength of Thalamic Projections to Area 21a

(1) LPI

Area 21a's strongest thalamic connection is with the LPI (*cortico-recipient zone*). Although this division does not receive a direct retinal projection, it does contain a retinotopic representation of the visual hemifield. This representation arises presumably from the cortical inputs which innervate this region from areas 17, 18, 19, 20a, 20b, 21a, 21b, AMLS, PMLS and VLS (Rosenquist, 1985). In particular, the LPI is the only major extrageniculate thalamic region to receive a massive topographical projection from area 17 (Berson and Graybiel, 1983). The anterograde uptake of HRP-WGA from the LPI injection indicates that the projection from the LPI to area 21a terminates in lamina 4 whilst a reciprocal projection arises from cells in lamina 6. This is the pattern expected for a loop that feeds forward from the LPI and back from area 21a to the LPI. Such a loop could be important in the exchange of information between area 21a and other visual areas (mentioned above) which have similar looping pathways through the LPI.

Experiments involving injections of the fluorescent tracers, FB and DY, into areas 17 and 21a, respectively, demonstrate that each of these two areas is connected with a topographically similar region of the LPI. The presence of separate though

overlapping populations of FB and DY labelled cells in the LPI indicates the close correspondence between the respective pathways which project from the LPI to areas 17 and 21a. This finding together with labelling of terminals in the LPI which resulted from anterograde transport of HRP-WGA and Biocytin from area 21a, suggests a possible model of information flow involving the striate cortex, the LPI and area 21a.

The finding that individual axons from cells in area 21a do not ramify into terminal arborisations but devolve, instead, into long thread-like processes that make *en passant* synaptic contact with a number of cells across the nucleus was a distinctive feature of Biocytin labelling in the LPI and is consistent with a possible model in which visual data is transferred between a number of cortical areas via the LPI.

Although area 21a appears to receive the bulk of its visual information via a pathway direct from the striate cortex (see Chapters 2, 3 and 5), additional or modified data from the striate cortex might also reach area 21a via the LPI. According to this model efferents from the striate cortex synapse in the LPI on cells which then project to area 21a. Whilst the results do not prove this to be the case, the findings are consistent with the speculation of Berson and Graybiel (1983) that the *striate recipient zone* of the LP-pulvinar complex figures prominently in the exchange of visual information between the striate and extrastriate cortex. In these circumstances, the role of the LPI would be to integrate visual information from the many cortical areas with which it is connected.

In examining the results from the present tracer experiments it was possible to address the question of the existence of a projection from the LPI to area 21a.

Although a separate population of labelled cells was not identified in the LPm following HRP-WGA injections of area 21a, consideration was given to the possibility that labelling in the LPI may extend into the LPm. Such a finding would be in accordance with that of Raczkowski and Rosenquist (1983) who found some retrogradely labelled cells in the LPm at caudal levels in the posterior thalamus following an HRP injection into area 21a and would indicate that visual data is reaching area 21a from the superior colliculus via the LPm (the *tectorecipient zone*). On the other hand, uncertainty about the existence of this pathway is exemplified by the fact that Symonds et al., (1981) failed to detect a projection to area 21a following bi-lateral injections into the LPm of [^3H] leucine. The present experiments, in which alternate HRP-WGA sections were reacted for the presence of AChE, however, add support to the view that an LPm-area 21a projection is non-existent.

(2) *Pulvinar*

A second pathway for information from the retina to area 21a could be via the pulvinar or *pretectorecipient zone*. The retina sends a major projection to the pretectum which, in turn, innervates the pulvinar (Berman, 1977; Graybiel, 1972a; Berson and Graybiel, 1978a). In addition, the pulvinar is the recipient of a direct projection from the retina (Berman and Jones, 1977) as well as an indirect one via area 19 (Kawamura et al., 1974; Updyke, 1977).

Unlike the geniculocortical system where the three parallel X-, Y- and W-type cell channels are present, the extra-geniculocortical system seems to contain only the W- and Y-type channels (Raczkowski and Rosenquist, 1983). These channels which represent separate, parallel streams have been less rigorously explored in the extrageniculate thalamus and there is uncertainty about the type of stream that projects to area 21a via the pulvinar. Nevertheless, the W- and Y-type

channels associated with the extrageniculate thalamus are probably highly implicated in visual processing performed in some regions of the association cortex.

Two observations shed light on the destination of geniculate streams in the cat. First, with bilateral lesions of area 17 and most of area 18, the cat retains good pattern vision but suffers a drop in spatial acuity (Berkely and Sprague, 1979). Second, area 17 is the only cortical area to receive X-type geniculate inputs and ablation of area 17 would selectively remove the X-type channel. Together, these two findings suggest that discrimination of pattern and shape is not wholly dependent on the X-cell channel and may be mediated by the paths supplying the extrastriate cortex. Supporting evidence for this proposal has also come from experiments in which lesions placed outside areas 17 and 18 (in areas 7, 19, 20, 21 and the lateral suprasylvian region) resulted in conspicuous deficits in pattern learning (Antonini et al., 1985).

These findings point to the possibility that Y- and W-type cells outside the primary visual cortex may contribute to pattern perception without aiding high acuity vision. For area 21a, the W and Y paths will be stronger in extrageniculate nuclei and, therefore, are likely to arise from the pulvinar. The present study, however, indicates a restricted spread of labelled cells in the pulvinar following retrograde axonal transport of tracers from area 21a. Therefore, as far as this cortical area is concerned, the results suggest that the strength of the pathway originating in the pulvinar may not be sufficient to subserve a visual function as comprehensive as pattern perception.

(3) LGNd

The results demonstrate that cells in the small cell C layer (layers C1-C3) of the LGNd send a projection to area 21a. Labelling, however, was not evident in every experiment and was never extensive which suggests that the projection may be quite weak. When labelled cells could be detected, the fact that they were located towards the medial edge of the LGNd which contains a representation of the central part of the visual field, is consistent with the retinotopic representation in area 21a (see Chapter 3). The result is also in line with evidence from other laboratories that the ventral C layers of the LGNd project to nearly all cortical visual areas (Raczkowski and Rosenquist, 1980).

The ventral C layers are the recipients of inputs principally from W-type cells (Cleland et al., 1976; Wilson et al., 1976) - one of the three streams of retinal ganglion cells which, together with X- and Y-type cells, remain segregated with little overlap in excitatory connections in the retinal-geniculate-cortical pathway (Sherman and Spear, 1982). The W-type cells comprise about half the population of retinal ganglion cells (Stone et al., 1979) and, although found throughout the retina, their density increases slightly along the visual streak running horizontally through the area centralis (Sherman and Spear, 1982). The visual streak is not as well developed in the cat's retina as in other species such as kangaroos and rabbits and an area centralis is found in association with a visual streak. Although the role of W-type cells in visual perception is not well understood, particularly as they respond sluggishly to visual stimuli (Cleland et al., 1973; Stanford et al., 1983), it has been suggested that they may play a role in the global modulation of the cortex (Kaas, 1986). The apparent weakness of the projection between the LGNd and area 21a suggests that the W-type cells make a minor contribution to the functioning of area 21a. Moreover, there has been speculation that projections, such as those emanating from the small cell C layers

of the LGNd, might reflect an interim organisational step that accompanies the transition from the tectum to the neocortex as the central processor for visual information (Diamond and Hall, 1969). Under these circumstances it would be possible that this pathway is a remnant of a prototypic visual system (Raczkowski and Rosenquist, 1980) and that its visual role has been superseded by the development of the neocortex.

(4) LGNv

The tracer experiments confirm that area 21a supplies information to the LGNv but does not appear to receive any input in return. The thalamo-cortical branch of this link was demonstrated by the presence of labelled terminals only in the LGNv following injection of area 21a with HRP-WGA and was the only example of a non-reciprocal thalamic pathway involving area 21a. Confirmation of the projection from area 21a to the LGNv came from the anterograde tracer experiments involving Biocytin. The results (particularly that illustrated in Fig.2.13) suggest that the LGNv is innervated from area 21a by large diameter axons which develop a profuse terminal arborisation. It was also apparent from the tracer experiments with Biocytin that there was a distinct difference in the labelling which occurred in the LGNv compared to that detected in other regions of the thalamus (see below).

Little is known about the role of the LGNv in vision. It is believed to receive some optic tract terminals (Hayhow, 1958; Laties and Sprague, 1966; Garey and Powell, 1968) but its major input is probably from the superior colliculus (Altman and Carpenter, 1961) and the LGNd (Altman, 1962). The results indicate that it has no direct impact on the cells of area 21a.

Terminals from Area 21a Projections to Different Thalamic Nuclei.

Labelled fibres of large diameter were more a feature of the projections to the LGNv than to other thalamic nuclei. For example, the LPI, pulvinar and LGNd were generally characterised by networks of fine processes although a fibre of greater diameter appeared occasionally in the LPI. The thicker diameter axons in the LGNv and the presence of profuse terminal arborisation, however, distinguished this nucleus from other labelled structures in the thalamus and might underlie its importance as a target of signals from area 21a. The existence of comparatively thick fibres in some thalamic nuclei may be indicative of the presence of myelination which characterises axons outside the cortex. These axons, which could often be traced for relatively long distances are presumed to be major descending projections from area 21a.

With respect to the size and character of boutons, however, there did not seem to be any marked differences amongst the projections to different thalamic nuclei. A schematic representation of the types of labelled processes which were found in the four labelled thalamic nuclei appears in Fig. 2.14.

Overview

In summary, these tracer experiments demonstrated the presence of reciprocal pathways between area 21a and the LGNd, pulvinar and LPI; and an efferent projection from area 21a to the LGNv. Within these thalamic nuclei some differences existed in the pattern of termination of axons projecting from area 21a. These differences were especially noticeable in the LPI and LGNv where they may reflect functional specialisation. Despite the earlier findings of ablation

experiments which demonstrated that a secondary or parallel pathway innervates the association cortex, there is considerable doubt whether thalamic pathways provide area 21a with visual information which parallels that which it receives from the primary visual cortex. Although connections with area 21a arose from neurons in the LGNd and pulvinar, both of which receive retinal inputs, they involved comparatively few cells and probably have little influence on the response properties of area 21a cells. As will be demonstrated in Chapter 5, the activity of cells of area 21a appears to be solely dependent on inputs from areas 17 and 18. Area 21a has a strong reciprocal connection with the LPI which terminated around cells projecting to the striate cortex. Thus two loops involving the LPI with areas 17 and 21a appear to arise from neighbouring cells although, so far, there is no evidence that the same cells contribute to both loops. Nevertheless, there exists the possibility that the LPI may be involved with the exchange of data between areas 17 and 21a.

Conclusion

The major conclusion drawn from this investigation is that, although area 21a receives afferent thalamic projections from the LGNd, pulvinar and LPI, those originating in the LGNd and pulvinar do not seem dense enough to determine the responses of area 21a cells (see Chapter 3). Area 21a's strongest thalamic connection is with the LPI (*cortico-recipient zone*) and this suggests that area 21a may be participating in a corticocortical loop via the LPI.

FIGURE 2.14

Biocytin labelling in several regions of the thalamus resulted from an injection of the tracer into area 21a. Figure 2.14 is a schematic representation of terminal arborisations found in these regions and are described briefly below.

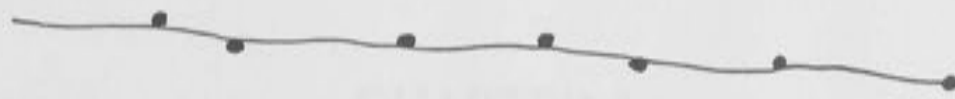
2.14(A) depicts an axon in the LPI with terminals protruding from its surface. Some of these axons were comparatively thick and could be traced for several hundred μm .

2.14(B) In the pulvinar, processes were usually quite fine and axon terminals were often located on short stalks which protruded from the axon.

2.14(C) Processes in the LGNd were also fine and, as was the case with the LPI, axon terminals were often situated on the surface of the axon.

2.14(D) Labelling in the LGNv differed from labelling in other regions of the thalamus. A single, large diameter axon appeared to innervate the region. This fibre broke down into finer processes which spread-out to form an extensive terminal arborisation.

(A)



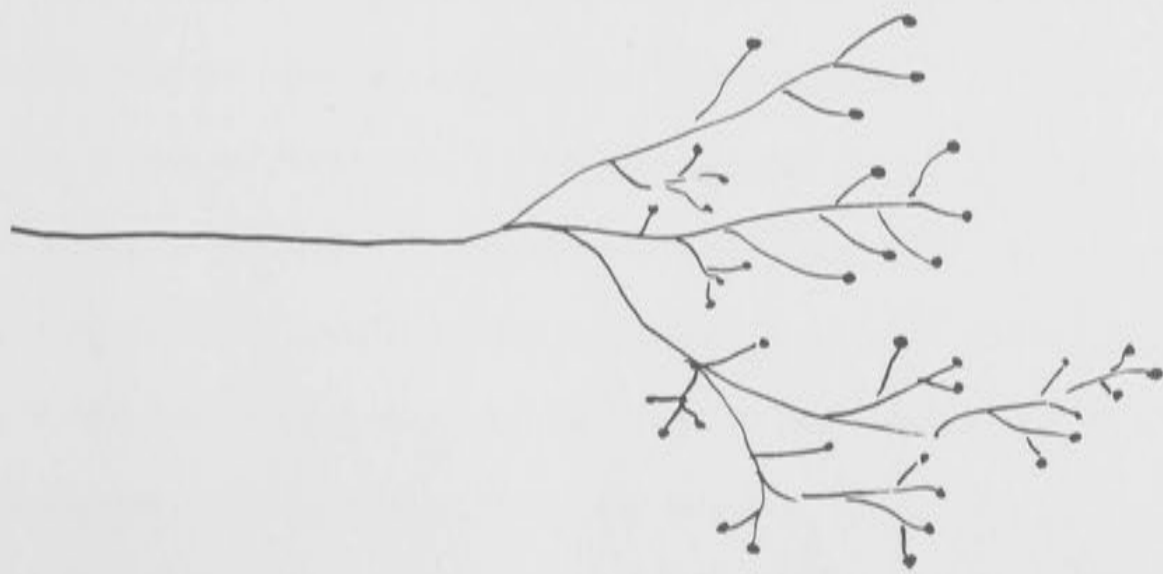
(B)



(C)



(D)



INTRODUCTION

CHAPTER 3

CORTICAL CONNECTIONS WITH AREA 21A

Much anatomical research in the last decade has been concerned with establishing connections between areas of the visual cortex which have been defined by retinotopic mapping. As a result of such mapping it has been suggested that the visual cortex contains distinct or more separate representations of the visual hemifield (Taub et al., 1974; Maunsell et al., 1975; Hubel and Livingstone, 1976). The existence of regions within the cortex that contain separate retinotopic representations suggest that such areas may be subserving a distinct aspect of visual perception. This is not to say, however, that such areas are necessarily specialised to perform a single visual function. It is possible that such areas or groups of areas act as a type of filter which modifies the visual information before relaying them to other regions. A variation of this view is that of Yaroslavskii et al. (1961) which proposes that groups or ensembles of cortical visual areas defined on the basis of the projections they receive from specific subdivisions of the LGN-pulvinar complex, may operate as functional systems.

INTRODUCTION

It has long been believed that functional localisation plays an important role in the organisation of the cerebral cortex. As a result areas of distinctive function have been defined on the basis of histochemical variations, differences in anatomical connections, topographical criteria, receptive field properties of neurons and behavioral differences following stimulation or inactivation (Rosenquist, 1985). The species most frequently used in these investigations have been monkeys and cats and it has been estimated that in the macaque monkey 60% of the cerebral cortex is dedicated to the processing of visual information compared to 30% in the cat (Orban, 1984).

Much anatomical research in the last decade has been concerned with exploring the connections between areas of the visual cortex which have been defined by retinotopic mapping. As a result of such mapping it has been concluded that the cat's visual cortex contains thirteen or more separate representations of the visual hemifield (Tusa et al., 1981; Mucke et al., 1982; Olsen and Graybiel, 1983). The existence of regions within the cortex that contain separate retinotopic representations suggest that each area may be contributing to specific aspects of visual perception. This is not to argue, however, that each area is uniquely specialised to perform a single visual function. It is possible that each area or group of areas acts as a type of filter which modifies the incoming visual signals before relaying them to other regions. A variation of this plan is that of Symonds et al., (1981) which proposes that groups or constellations of cortical visual areas, defined on the basis of the projections they receive from specific sub-divisions of the LP-pulvinar complex, may operate as functional sub-units.

A number of anatomical investigations with axonal tracers has revealed that areas of the visual cortex are interconnected by a complex pattern of neural pathways (Symonds and Rosenquist, 1984a; Van Essen, 1985; Bullier, 1986; Dreher, 1986; Ungerleider and Desimone, 1986). In order to reach an understanding of the nature of visual processing it is necessary to possess a detailed knowledge of how these pathways interconnect. For example, an appreciation of the sources of signals reaching a specific area and the destination of signals it transmits may contribute to an understanding of the functional significance of the area.

Whilst we now possess a good generalised picture of corticocortical connections in the cat, considerably less detailed information is available concerning the laminar distribution of these connections. Henry et al., (1990) have drawn attention to the difficulties in interpretation caused by inconsistencies in data published on the laminar distribution of terminals and the spatial extent of corticocortical connections. The problem is exacerbated when the axons originate from neurons in a small area of cortex.

It seems likely that an important determinant of the pattern of laminar connectivity is the type and function of the commonest resident neuron. The cells of the visual cortex have been categorised into separate classes on the basis of morphological, physiological and biochemical differences but these classes are not distributed randomly across all layers of the cortex and are more likely to be in greater numbers in one or more laminae (Symonds and Rosenquist, 1984b). For example, spiny stellate cells are found only in lamina 4 of cats and monkeys (Garey, 1971; LeVay, 1973; Lund et al., 1979) whilst chandelier cells predominate in laminae 2/3 of cats and rats (Somogyi, 1977; Fairen and Valverde, 1980; Peters et al., 1982). Moreover, since there is evidence that cells in different laminae display different receptive field characteristics (Hubel and Wiesel, 1962; Gilbert, 1977; Leventhal and Hirsch, 1978),

it would seem very likely that there is a correlation between cell type, laminar location and functional properties.

Bullier et al., (1984b) have pointed-out that a possible way to create functional specialisation is to segregate the different types of information circulating in the cortex by separating the neurons participating in different corticocortical loops into different cortical laminae. Their investigations provided support for such a proposition when they found in the cat, that cortical projections arise from cells in supragranular laminae 2/3 and in infragranular laminae 6. Differences in the proportion of cells present at each laminar level depend mainly on the area of origin involved in the connection. For example corticocortical feedforward projections from lower levels of the cortical hierarchy such as areas 17 and 18 tend to arise from the supragranular laminae whereas feedback connections from higher to lower areas arise mainly from cells in the infragranular laminae. These results provided a sound basis for a model of information processing in the cortex in which functional specialisation is dependent on specific neuronal circuits.

In the present study, the specialised response properties of the neurons of area 21a which are considered in Chapters 3, 4 and 5, may be reflected anatomically by the segregation of area 21a's cortical loops into different cortical laminae. In this chapter as well, therefore, an attempt is made to identify individual patterns of connectivity at both regional and laminar levels. From the underlying structural organisation revealed in area 21a it should be possible to arrive at a better understanding of the region's functional contribution to visual processing.

MATERIALS AND METHODS

Processing of sections with respect to cutting, staining and mounting was identical to that in Chapter 2 with the exception that no AChE histochemistry was undertaken on cortical slices.

Animal preparation

The experiments were performed on adult cats, weighing between 2.0 and 2.5 kg. Surgical procedures and injections of HRP-WGA and Biocytin into areas 21a and 17 were identical to those described in Chapter 2.

In addition, paired injections of fluorescent tracers were placed in area 21a to investigate whether two segregated populations of neurons within both area 17 and area 18 provide inputs to separate sub-regions within area 21a. Diamadino yellow (DY) and Fast blue (FB) were injected into different regions of area 21a (separated by 2mm) by means of a Hamilton syringe. The concentrations and preparation of each tracer followed the procedure described in Chapter 2. The DY injection was placed at HC: -1.0mm anteroposterior and 12.0mm mediolateral. FB was injected at HC: -3.0mm anteroposterior and 12.0mm mediolateral. Both injections were made 1.5mm below the cortical surface and the syringe was tangentially oriented at 10°. Special care was taken to ensure that the separate integrity of the injections remained intact and subsequent inspection of the injection sites showed no evidence of cross-contamination.

Perfusion and fixation

Perfusion and fixation procedures applicable to HRP-WGA, Biocytin and the fluorescent tracers were identical to those described in Chapter 2.

Preparation of Histological Sections RESULTS

Processing of sections with respect to cutting, staining and mounting was identical to the procedures set-out in Chapter 2 with the exception that no ACh.E histochemistry was undertaken on cortical tissue.

Connections with the Ipsilateral Visual Cortex

Eight cats received injections of the neuronal tracer, DAPI-WGA into area 17a. As a result of these injections, labelled cell bodies and axon terminals were evident in ten areas of the visual cortex in the ipsilateral hemisphere. These areas were 17, 18, 19, 20a, 20b, 21b, DLS (dorso-lateral supravivian), PLLS (posterior-medial lateral supravivian), PLIS (posterior-lateral lateral supravivian) and PS (posterior supravivian). Labelling was also present in area 7, a region usually associated with visual processing, but one that differs from other visual cortical areas in being polyfunctional (Riesenhuber, 1985).

Drawing tube illustrations in Fig. 3.1 of a series of 13 coronal sections of the ipsilateral cortex show the pattern of cortical areas with area 17a, which is the labelled area, the region of filled cells and terminals, and area 17b, a portion of the cortex which is known to receive a retinotopic representation of the visual field (see Palmer et al., 1978; Tusa and DeYoe, 1980; Tusa et al., 1980, 1979; 1981; Ungerleider, 1975). The most caudal labelling was observed in the supragranular or infragranular laminae and was observed in every section. The sections in Fig. 3.1 range in distance from HC posteriorly. AP is indicated anteroposterior (AP) with each section being 1 mm thick. The designation of cortical areas in this and later illustrations are in accordance with diagrams of cortical regions presented by Tusa and DeYoe (1980) and Riesenhuber (1985).

RESULTS

HRP-WGA Labelling

Connections with the Ipsilateral Visual Cortex

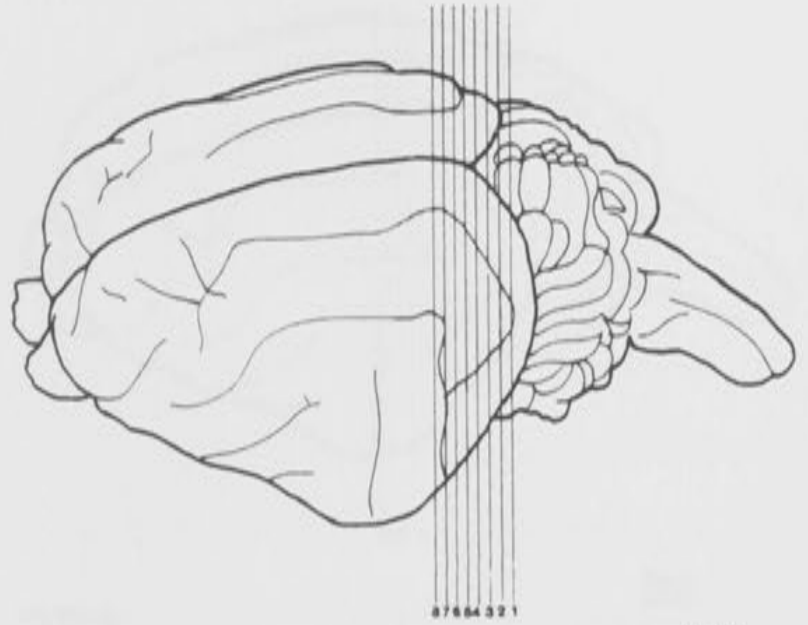
Eight cats received injections of the axonal tracer, HRP-WGA into area 21a. As a result of these injections, labelled cell bodies and axon terminals were evident in ten areas of the visual cortex in the ipsilateral hemisphere. These areas were 17, 18, 19, 20a, 20b, 21b, DLS (dorsal-lateral suprasylvian), PMLS (posterior-medial lateral suprasylvian), PLLS (posterior-lateral lateral suprasylvian) and PS (posterior suprasylvian). Labelling was also present in area 7, a region mainly concerned with visual processing, but one that differs from other labelled cortical areas in being polyfunctional (Rosenquist, 1985).

Drawing tube illustrations in Fig. 3.1 of a series of 13 coronal sections of the ipsilateral cortex show the nature of cortical links with area 21a. Within most labelled areas the region of filled cells and terminals was localised in a part of the cortex which is known to contain a retinotopic representation of the central part of the visual field (see Palmer et al., 1978; Tusa and Palmer, 1980; Tusa et al., 1978; 1979; 1981; Updyke, 1977). In most cases labelling was restricted to either the supragranular or infragranular laminae and was heaviest in areas 17 and 18. The sections in Fig. 3.1 range in location from HC co-ordinates: -8.0 to +3.0mm anteroposterior (AP) with each section being 1.0mm displaced from its neighbour. The designation of cortical areas in this and later illustrations are referenced to diagrams of cortical regions contained in Symonds and Rosenquist (1984a) and Rosenquist (1985).

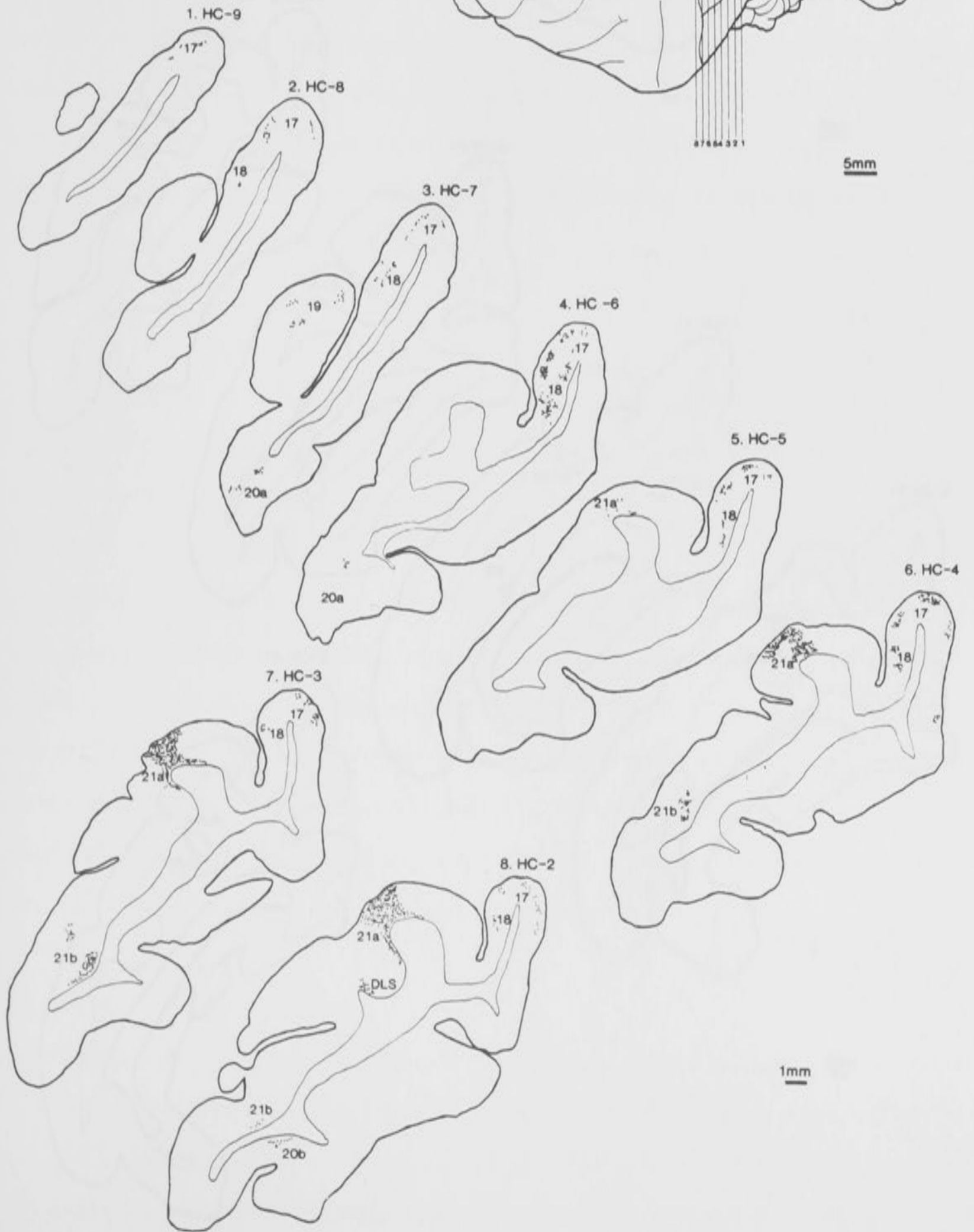
FIGURE 3.1

Drawing tube illustrations of a series of 13 coronal sections through the cortex depicting areas which were labelled as a result of an HRP-WGA injection into area 21a. The sections, which are separated by a distance of 1mm, range from HC -9.0 to +3.0mm anteroposterior (AP). The insets indicate the AP location of each section.

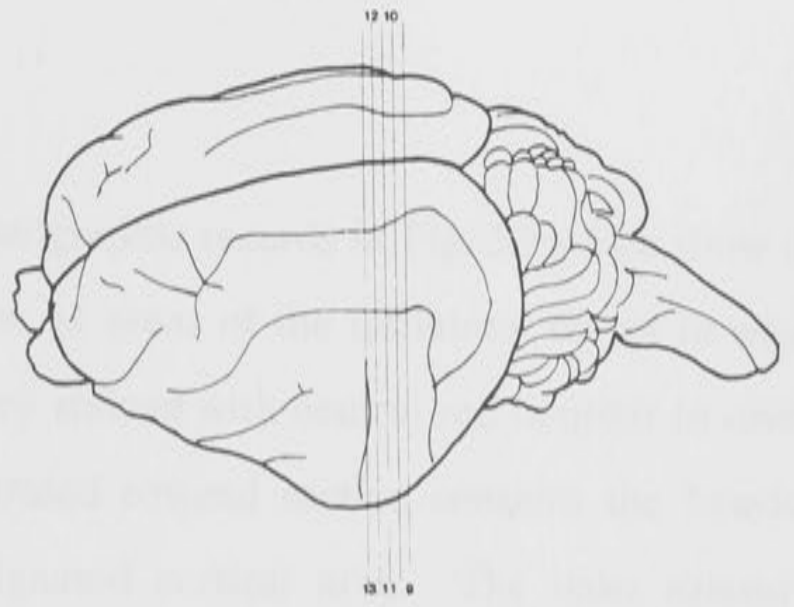
Labelling of cells and axon terminals was present in areas 17, 18, 19, 20a, 20b, 21b, DLS, PMLS, PLLS, PS and 7. It was located in regions known to contain a retinotopic representation of the central part of the visual field and was usually situated in either the supragranular or infragranular laminae..



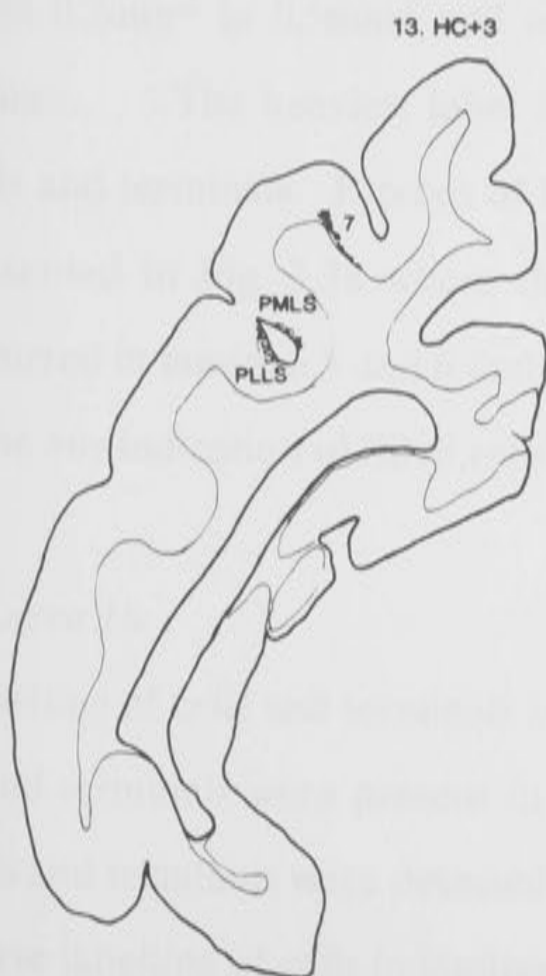
5mm



1mm



5mm



1mm

The same findings are presented as photographic records in Fig. 3.2 which show the distribution of cells and terminals in the 11 areas of the ipsilateral cortex in which labelling was detected. The sections were stained with neutral red in order to reveal the pattern of lamination. Each illustrated coronal section contains the heaviest labelling found for the particular designated cortical area. The most extensive labelling occurred in the primary visual cortex (areas 17 and 18) whilst comparatively sparse labelling was observed in other areas of the cortex. A detailed analysis of the results is presented in the following survey.

(1) Area 17

The striate cortex contained evidence of widespread, dense labelling. It extended from HC: -9.0 to 0.0mm AP. In lamina 1 labelled boutons were evident as a layer of dust-like particles but there was an absence of any filled cells. The heaviest labelling was often confined to patches of cell bodies and terminals which appeared periodically in laminae 2/3 adjacent to the pial surface. The patches varied in size from 0.2mm^2 to 0.5mm^2 and were separated by distances of between 0.3mm and 1.2mm. The heaviest label in laminae 2/3 involved small to medium pyramidal cells and terminals. Patches of labelled cells are visible in the dark field illustration presented in Fig. 3.3a where they are indicated by arrows. Sparse labelling also occurred in laminae 5 and 6 and is apparent in Fig. 3.2. However, there did not seem to be any indication of filled cells or terminals in lamina 4.

(2) Area 18

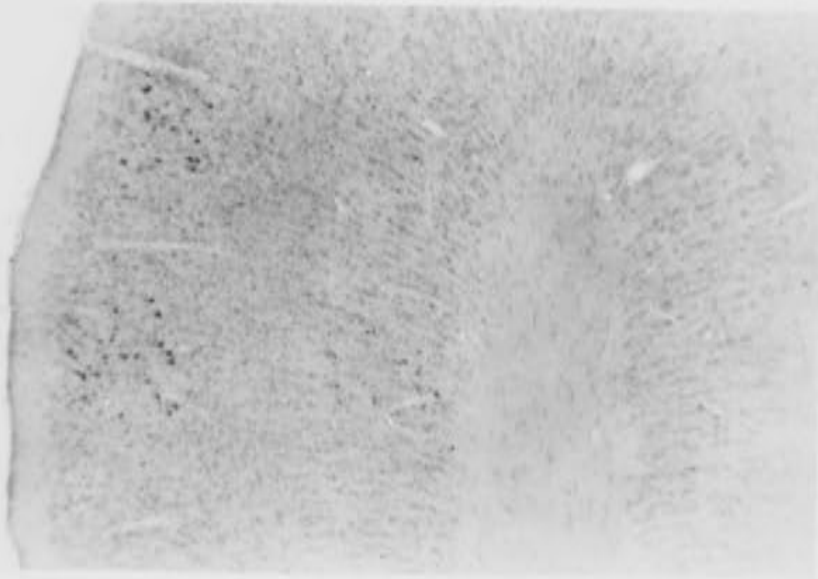
Labelling of cells and terminals in area 18 extended from HC: -8.0mm to -2.0mm AP. Filled terminals were present in lamina 1 whilst in laminae 2/3 both densely filled cells and terminals were detected. As was the case with area 17, there was also some sparse labelling of cells in laminae 5 and 6 but lamina 4 did not appear to contain any

FIGURE 3.2

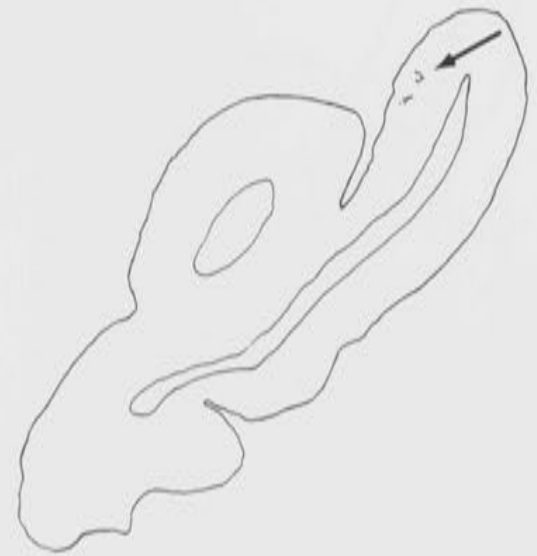
Photomicrographs of the 11 areas of the cortex where HRP-WGA labelling was found following an injection of the tracer into area 21a. The sections were stained with neutral red in order to reveal the pattern of cortical lamination. Filled cells appear as dark dots but axon terminals are not visible at this magnification. Beside each illustration is a drawing of a coronal section of cortex relevant to the location of the photomicrograph with arrows indicating the position of labelling.

In areas 17 and 18 labelled cells are evident in the supragranular and infragranular laminae whilst in area 19 of this illustration they appear only in the supragranular lamina. In areas 20a, 20b, 21b, PMLS, PLLS, DLS, 7 and PS the labelling was most evident in the infragranular laminae.

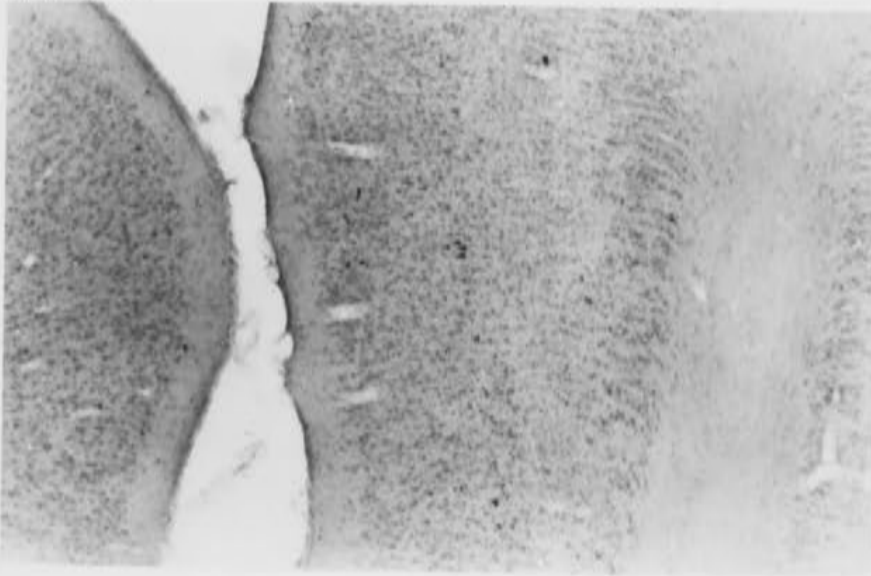
AREA 17



HC -6.50



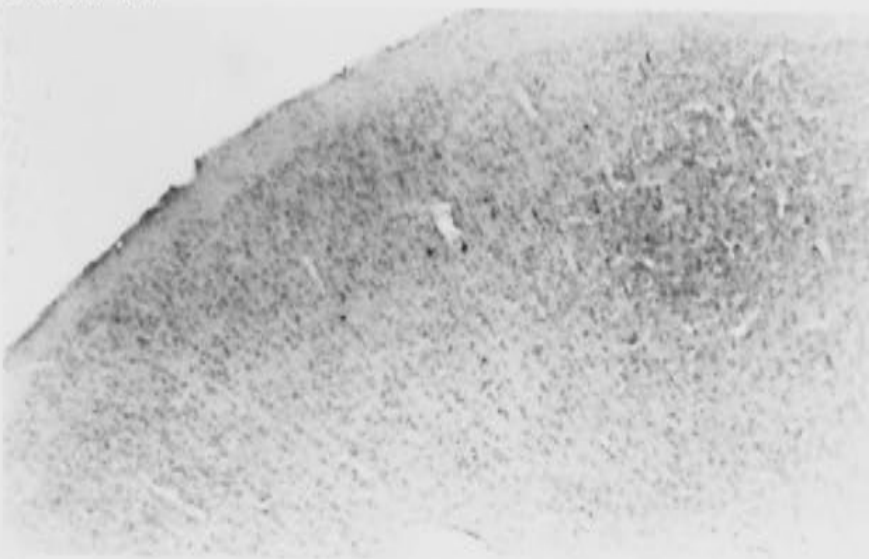
AREA 18



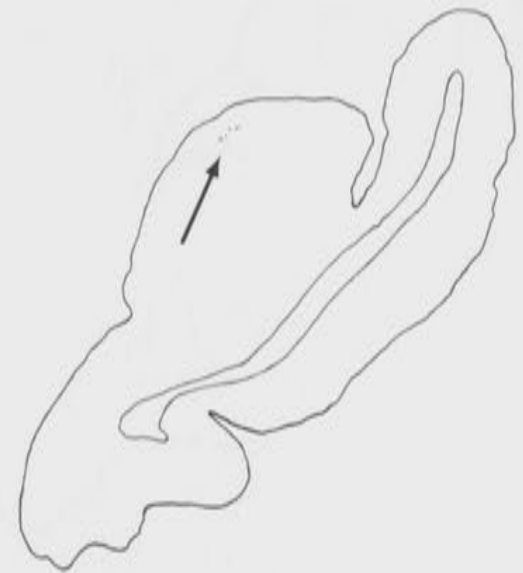
HC -6.00



AREA 19



HC -6.25



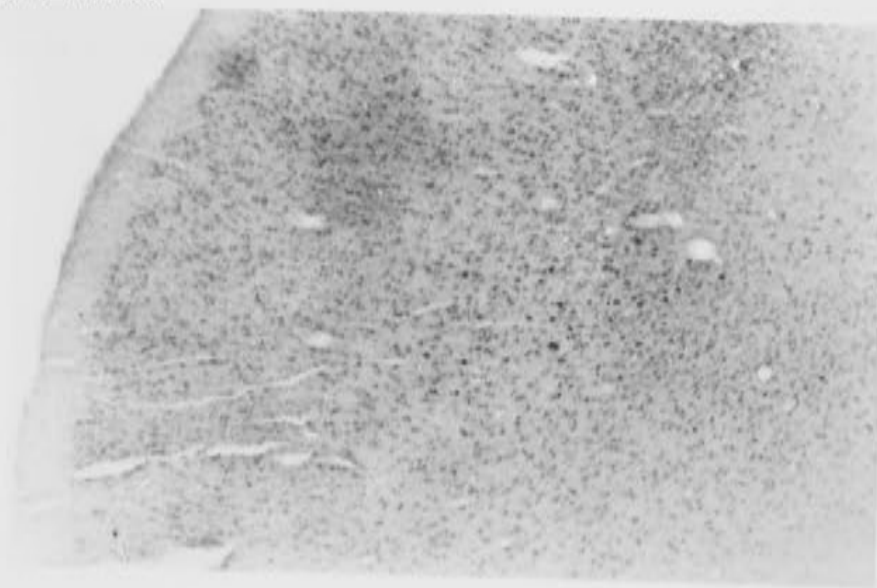
1mm



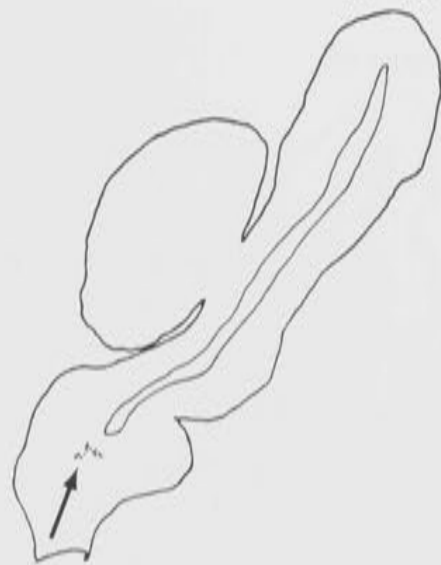
5mm



AREA 20a



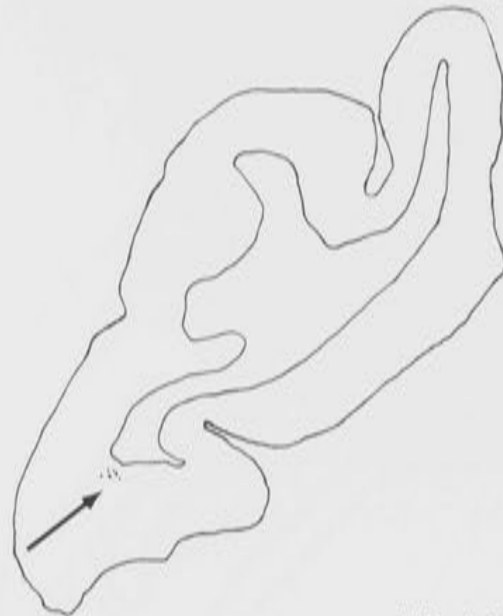
HC -7.00



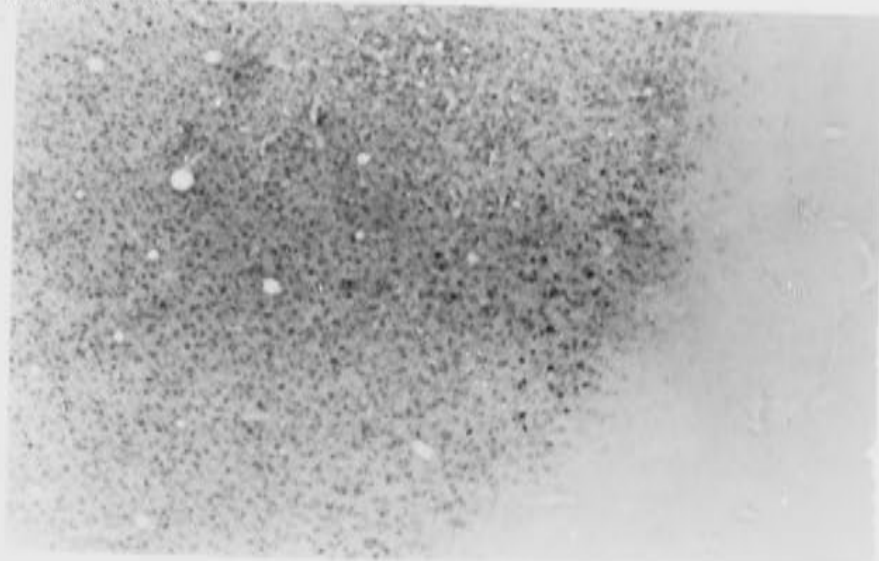
AREA 20b



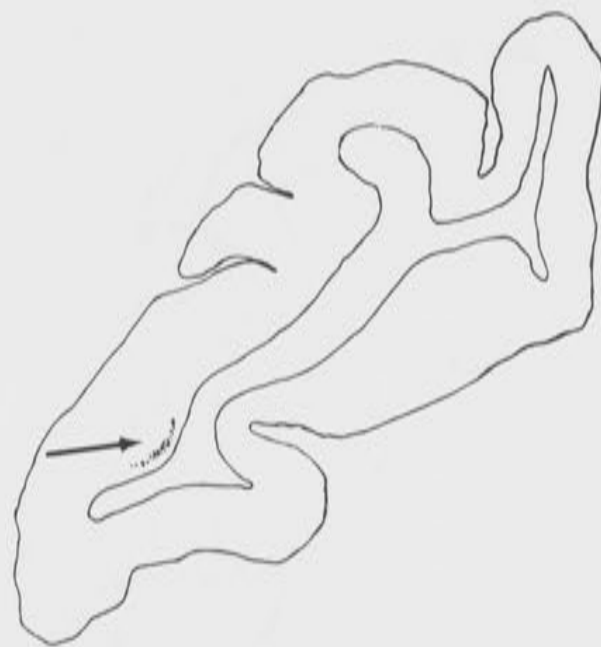
HC -5.75



AREA 21b



HC -3.50



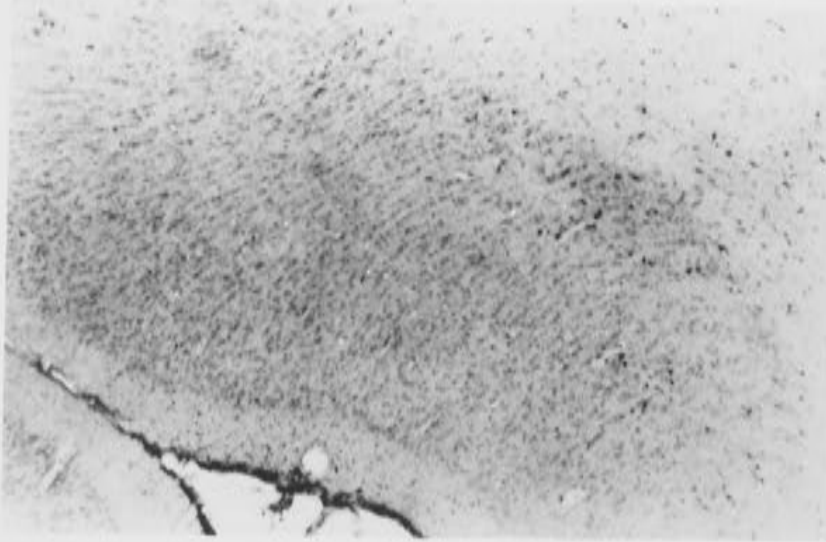
1mm



5mm



AREA PMLS



HC +2.00



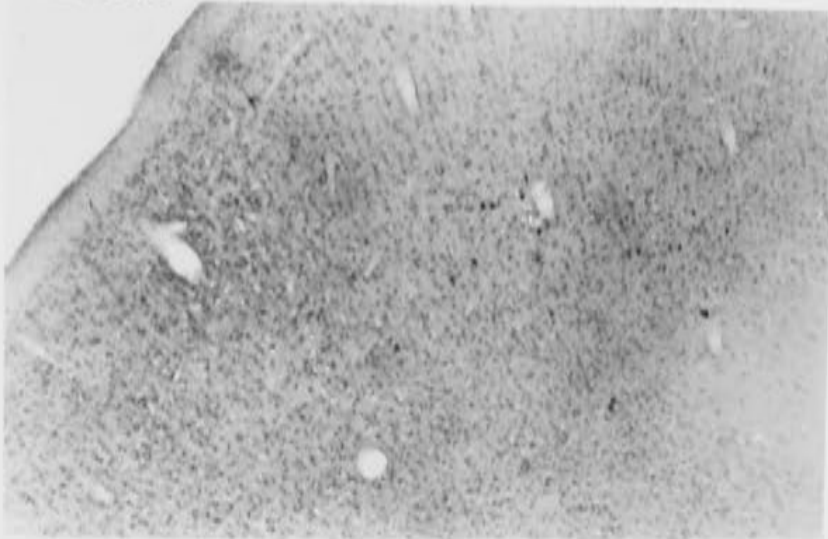
AREA PLLS



HC 0.00



AREA DLS



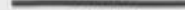
HC -1.00



1mm



5mm



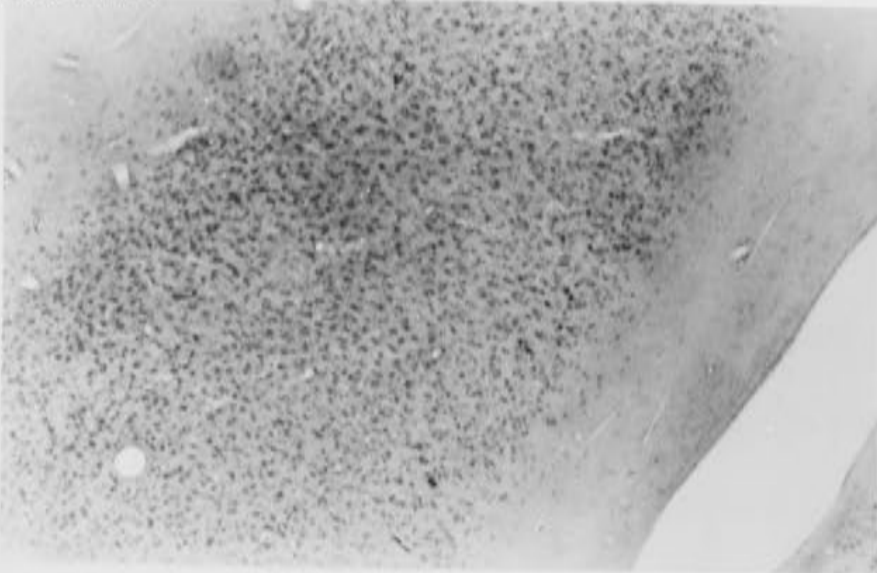
AREA 7



BWP16
HC +3.00



AREA PS



HC +1.00



1mm



5mm



labelling. Under the light field photomicrograph in Fig. 3.2 the labelling appears to be sparse and scattered but often it was found in patches. For example, Figs. 3.3b and 3.3d present dark field photomicrographs of two separate labelled patches in laminae 2/3 of area 18. Although the patches were less numerous than in area 17, their dimensions and distances apart were similar to those present in the striate cortex.

(3) Area 19

Figure 3.2 shows a coronal section with a few labelled cells and terminals in area 19 that extended from HC: -7.0 to +1.0mm AP. HRP-WGA labelling in this area was not heavy although it was widespread throughout the region. In common with the labelling in areas 17 and 18 it was sometimes restricted to patches of cells. This is better depicted in the darkfield photomicrograph in Fig. 3.3c where arrows show the location of two labelled patches. The patchiness in area 19, however, was often less distinct than in the striate cortex. Terminals in lamina 1 were labelled although this was not as dense as in areas 17 and 18. Labelled cells and terminals were present in both the supragranular and infragranular laminae but, unlike labelling in areas 17 and 18, the populations in each laminar division seemed to be of similar proportion to one another. Labelled cells in lower lamina 3 may have spread into lamina 4 but this could not be verified with certainty. The darkfield photomicrograph presented in Fig. 3.3d shows another patch of labelling in area 19 as well as labelling in area 18. Both patches were located in the supragranular laminae and are separated from each other by the lateral sulcus.

(4) Association Cortex

Labelling of both cell bodies and axon terminals could be detected in several areas of the ipsilateral association cortex but it was generally not as heavy as that which occurred in the primary visual cortex and it was most often located in the infragranular laminae. The areas which were labelled were 20a, 20b, 21b, PMLS, PLLS, DLS, 7 and PS. Labelling was usually evenly diffuse and the presence of separate patches of filled cells and axon terminals, which was a prominent feature of labelling in areas 17 and 18, could not be detected in any area of the association cortex. The patchiness, therefore, appeared to be a characteristic of projections arising from the supragranular laminae. Drawing tube illustrations of labelling in the association cortex is shown in Fig. 3.1.

Labelling in areas 20a, 20b, 21b and PS is also presented in the series of photomicrographs in Figure 3.3. Beside each photograph a drawing tube illustration shows the relevant coronal section with the heaviest labelling. Arrows mark the location of the labelling and the HC anteroposterior co-ordinates are recorded for each section. The final photomicrograph in Fig. 3.3 (designated area PS) presents an example of labelled cells and terminals that were evident in a zone of the cortex close to the lateral ventricle. This region was situated on the lateral side of the brain at HC: +1.00mm AP. The labelling can be seen in lamina 6 adjacent to the white matter which lies between the lamina and the lateral ventricle. The labelled region is believed to be part of the posterior suprasylvian area (PS).

Laminar Disposition of Extrinsic Projecting Cells

In both areas 17 and 18 the number and density of labelled patches in the infragranular laminae was considerably less than in the supragranular laminae. The

FIGURE 3.3

Darkfield photomicrographs of HRP-WGA labelling in seven areas of the cortex. Arrows indicate the regions of labelled cells.

Figs. 3.3(A) to 3.3(D) show regions in areas 17, 18 and 19 which contained the heaviest labelling. Labelling of axon terminals in lamina 1 of areas 17, 18 and 19 appears as a light strip along the pial edges of the sections. Patches of filled cells are evident in the supragranular laminae of these areas.

Figs. 3.3(E) to 3.3(H) show regions of labelling in the infragranular laminae of areas 20a, 20b, 21b and PS. This labelling, however, did not occur in patches.

(A) AREA 17



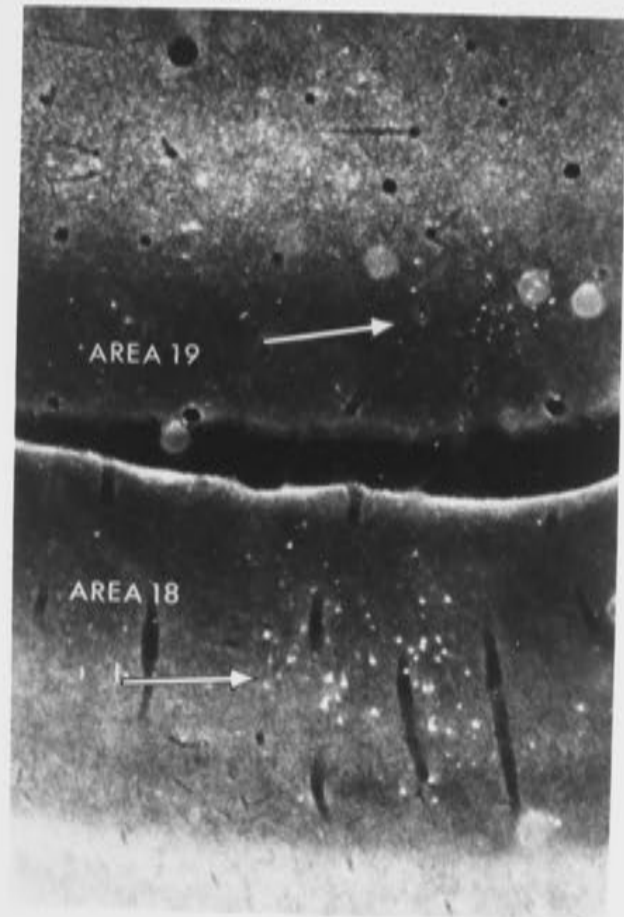
(B) AREA 18



(C) AREA 19



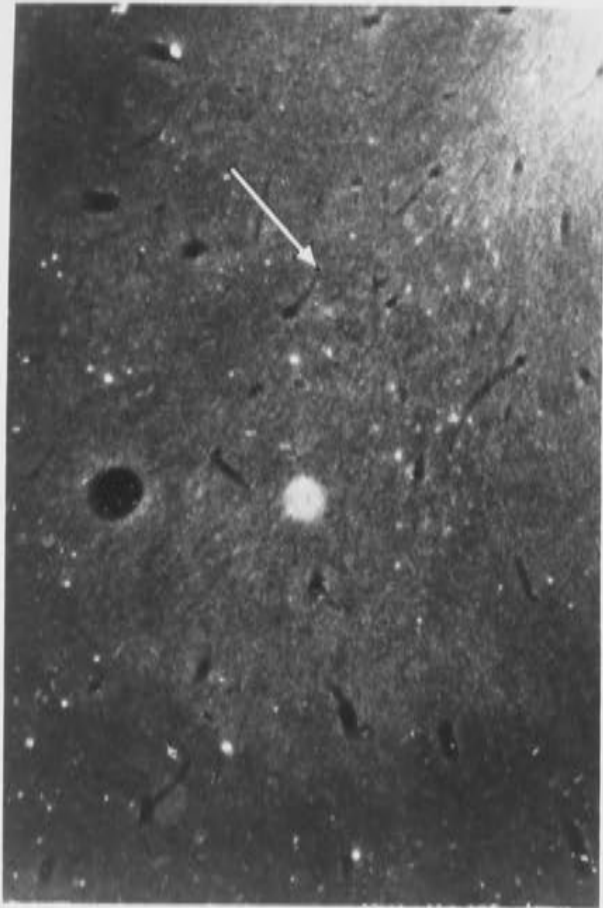
(D) AREAS 18 & 19



1mm



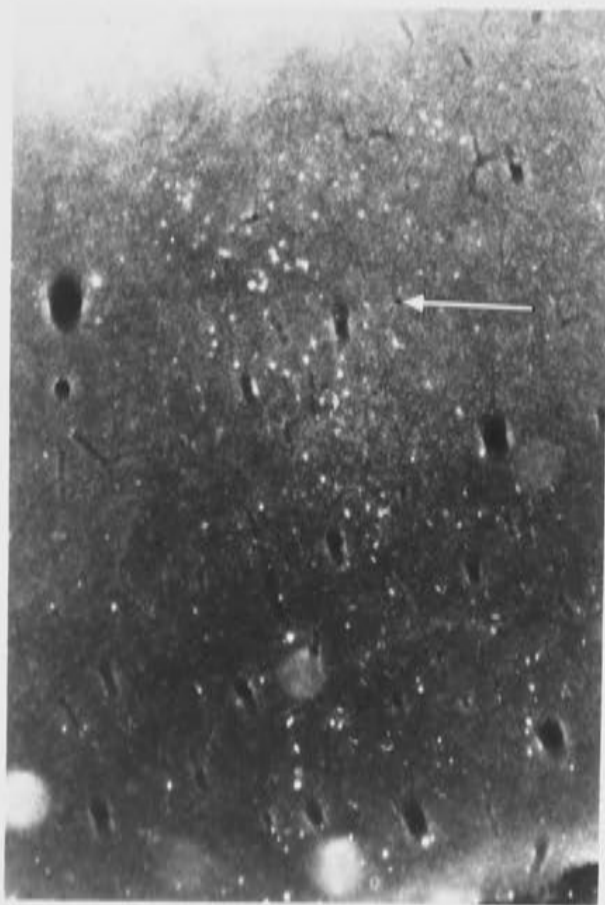
(E) AREA 20a



(F) AREA 20b



(G) AREA 21b



(H) AREA PS



1mm

labelling at both levels, however, frequently appeared to lie in the same vertical plane through the cortex. Labelling of both laminar levels was especially apparent in area 17 and is illustrated in Fig. 3.4 which is part of a coronal section through the striate cortex extending from the white matter to the pial surface. The tissue was stained with neutral red to reveal the pattern of lamination. In this illustration, the cluster of pyramidal cells in laminae 2/3 is matched by a small corresponding patch of cells in lamina 6 lying in the same vertical plane.

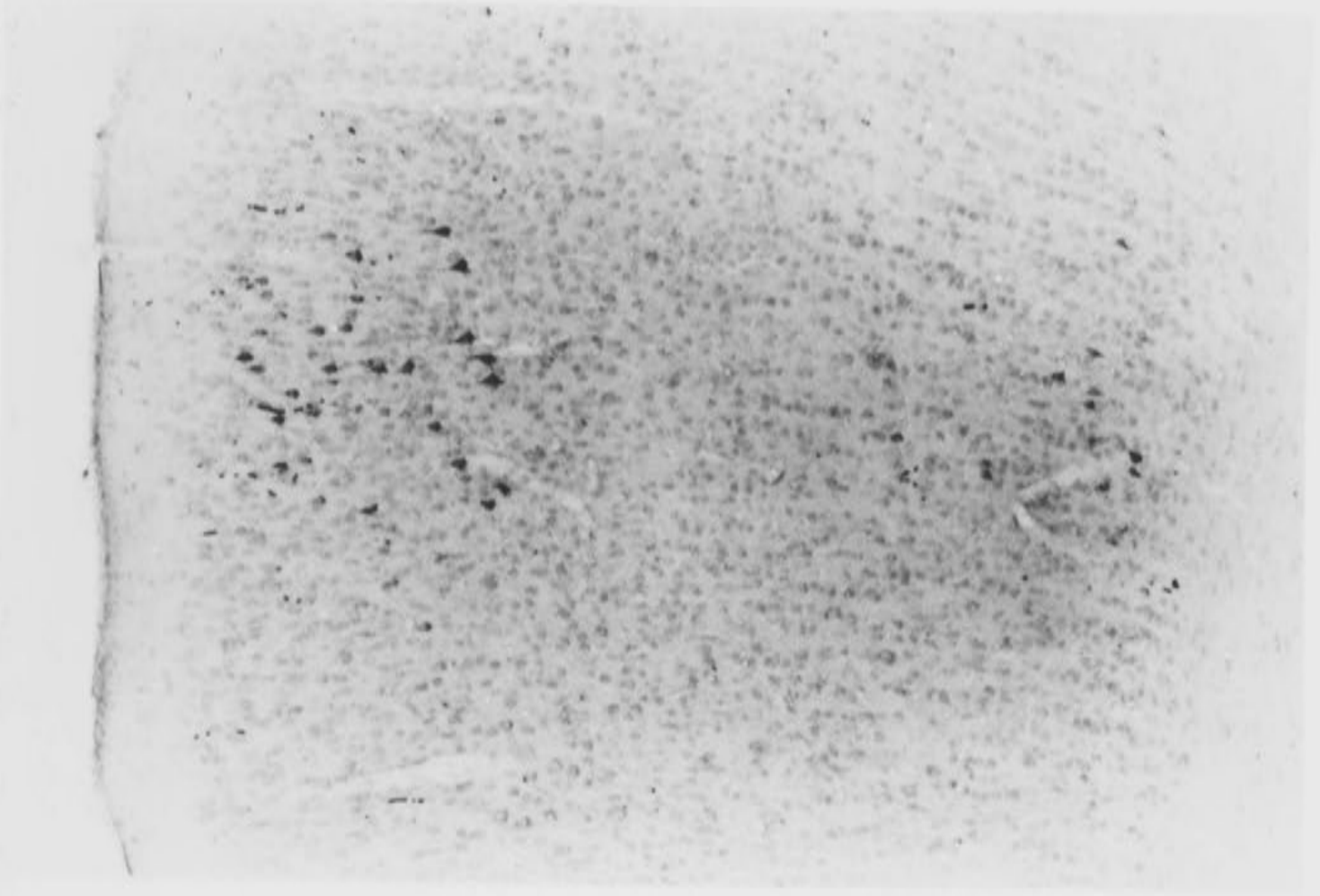
Where patches of labelling were visible at both the supragranular and infragranular levels of area 17, the numbers of labelled cells were counted for a sample of 8 regions and a comparison made of the relative density of labelled cell populations at the two levels. Fig. 3.5(A) presents drawings of the eight regions. These drawings were first prepared through a drawing tube and then transferred to a Tectronix Interactive Digital Plotter where the relative positions of the cells were mapped and cell counts were performed. Stippled areas indicate the clusters of HRP-WGA labelled cells and the dashed lines designate the divisions between laminae 1, 2/3, 4 and 5/6. Histograms showing the relative laminar distribution of the labelling in the eight samples is presented in Fig. 3.5(B). The total number of cells counted was 286 of which 81% were located in the supragranular laminae and 19% in the infragranular laminae.

In area 18 the relative densities of labelled cells and terminals in the supragranular and infragranular laminae seemed similar to those present in the striate cortex. In area 19, however, labelling of cells and terminals appeared to be evenly distributed at both laminar levels.

In contrast to areas 17 and 18 where the heaviest labelling was found in the supragranular laminae, the densest labelling of cells and terminals in areas of the

FIGURE 3.4

A photomicrograph of a segment of a coronal section through the striate cortex extending from the pial surface to the white matter showing the presence of labelled cells which resulted from an HRP-WGA injection into area 21a. The section was stained with neutral red to reveal the pattern of cortical lamination. A large patch of labelled cells is present in lamina 2 and upper lamina 3. In addition a few filled cells are visible in laminae 5 and 6 in the same plane as the labelling in the supragranular laminae.



1mm

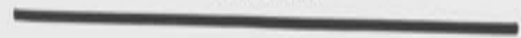
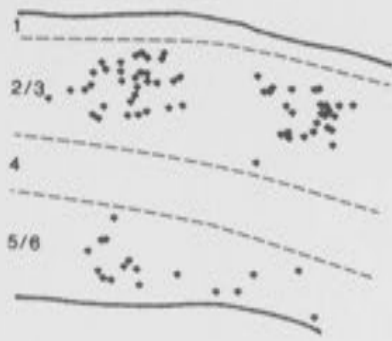


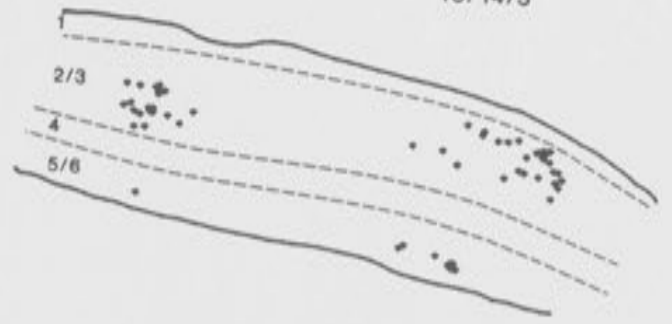
FIGURE 3.5(A)

Drawings of eight regions of the striate cortex indicating the relevant laminar location of HRP-WGA labelled cells resulting from an injection of the tracer into area 21a. Stippled areas indicate clusters of labelled cells and the dashed lines designate the main laminar divisions. Note that most labelling was found in the supragranular laminae and often occurred in patches.

16/14/1



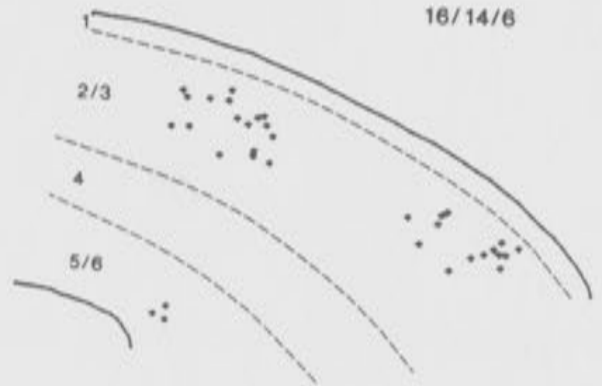
16/14/5



16/14/2



16/14/6



16/14/3



16/14/7



16/14/4



16/14/8



1mm

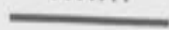
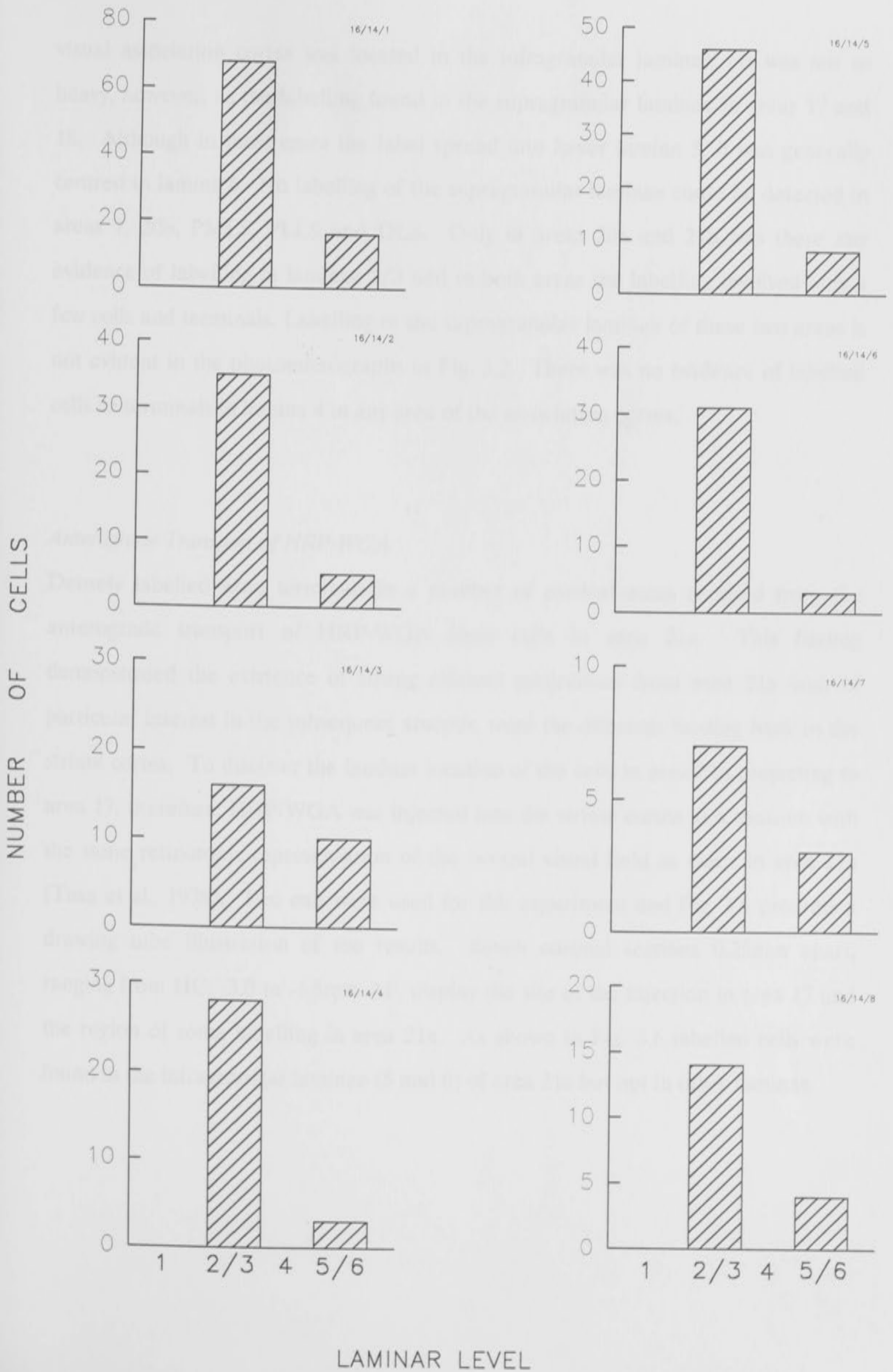


FIGURE 3.5(B)

Eight histograms derived from the sample data contained in the previous figure showing the distribution of HRP-WGA labelled cells in laminae 2/3 and 5/6 of the striate cortex. In this figure, a total of 286 cells were counted, of which 81% were located in the supragranular laminae (2/3).



visual association cortex was located in the infragranular laminae. It was not as heavy, however, as the labelling found in the supragranular laminae in areas 17 and 18. Although in most cases the label spread into lower lamina 5, it was generally centred in lamina 6. No labelling of the supragranular laminae could be detected in areas 7, 20b, PMLS, PLLS and DLS. Only in areas 20a and 21b was there any evidence of labelling in laminae 2/3 and in both areas the labelling involved only a few cells and terminals. Labelling in the supragranular laminae of these two areas is not evident in the photomicrographs in Fig. 3.2. There was no evidence of labelled cells or terminals in lamina 4 in any area of the association cortex.

Anterograde Transport of HRP-WGA

Densely labelled axon terminals in a number of cortical areas resulted from the anterograde transport of HRP-WGA from cells in area 21a. This finding demonstrated the existence of strong efferent projections from area 21a and, of particular interest in the subsequent analysis, were the efferents feeding back to the striate cortex. To discover the laminar location of the cells in area 21a projecting to area 17, therefore, HRP-WGA was injected into the striate cortex at a location with the same retinotopic representation of the central visual field as exists in area 21a (Tusa et al., 1978). Two cats were used for this experiment and Fig. 3.6 presents a drawing tube illustration of the results. Seven coronal sections 0.25mm apart, ranging from HC: -3.0 to -1.5mm AP, display the site of the injection in area 17 and the region of soma labelling in area 21a. As shown in Fig. 3.6 labelled cells were found in the infragranular laminae (5 and 6) of area 21a but not in other laminae.

FIGURE 3.6

Drawing tube illustrations of seven coronal sections through the cortex showing the location of an HRP-WGA injection into area 17 (hatched region) and the resulting labelling of cells in area 21a (stippled region). A dotted line indicates the area 17/18 boundary. The sections extend from HC -3.0 to -1.5mm AP and each is separated by a distance of 0.25mm. The inset indicates the relative position in the cortex of each coronal section. Note that labelling of cells in area 21a is located in the infragranular laminae.

Summary of FFA-1024 Labeling in the Entorhinal Cortex

The anatomical positions in case 7 are as follows: 1. HC -3.0, 2. HC -2.75, 3. HC -2.50, 4. HC -2.25, 5. HC -2.00, 6. HC -1.75, 7. HC -1.50

Cells projecting to area 17 and 18

Figure 1

Coronal section

of the entorhinal cortex

showing the location of the

microprobe

used for the injection of

the tracer

into the entorhinal cortex

at various depths

from the surface

to the white matter

border

of the entorhinal cortex

at various depths

from the surface

to the white matter

border

of the entorhinal cortex

at various depths

from the surface

to the white matter

border

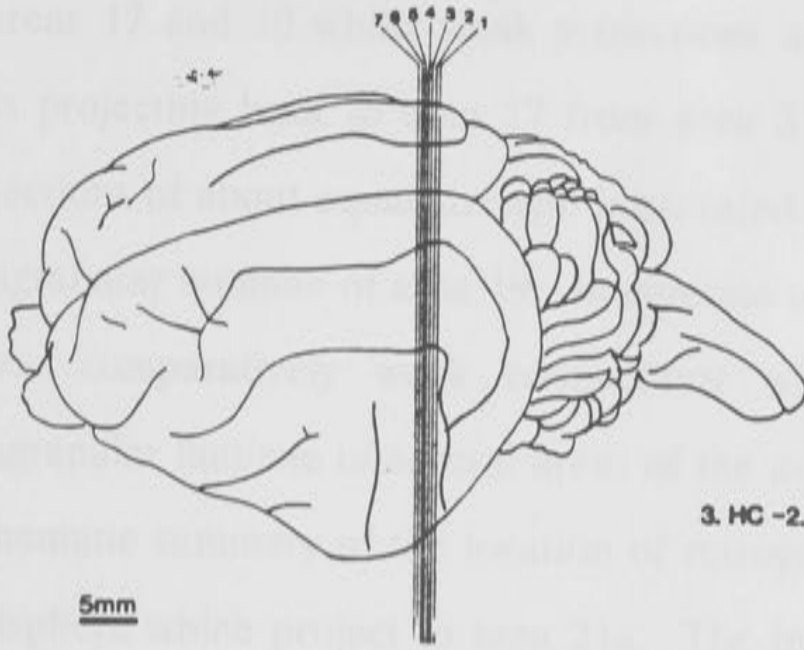
of the entorhinal cortex

at various depths

from the surface

to the white matter

border



5mm

1. HC -3.0

2. HC -2.75

3. HC -2.50

4. HC -2.25

5. HC -2.00

6. HC -1.75

7. HC -1.50

1mm

..... AREA 17/18 BORDER

Summary of HRP-WGA Labelling in the Ipsilateral Cortex

The strongest projections to area 21a arose from cells in the supragranular laminae of areas 17 and 18 whilst weak projections arose from the infragranular laminae. Cells projecting back to area 17 from area 21a were located in laminae 5 and 6. Projections of about equal strength innervated area 21a from the supragranular and infragranular laminae of area 19. In contrast to projections from the primary visual cortex, comparatively weak connections with area 21a emanated from the infragranular laminae of several areas of the association cortex. Figure 3.7 provides a schematic summary of the location of retrogradely labelled cells in the ipsilateral hemisphere which project to area 21a. The inset diagram summarises the laminar pattern of projections from area 21a to area 17..

Biocytin Labelling

The unsuccessful application of Pha-L as an anterograde axonal tracer, as was mentioned in Chapter 1, led to experiments using Biocytin. This tracer was used to reveal the destination and laminar location in the ipsilateral cortex of axons projecting from the cells of area 21a. Four cats were used in this experiment, in which area 21a was injected with Biocytin. Figure 3.8 presents a drawing tube illustration of a typical Biocytin injection site. Filled processes were especially evident in the supragranular laminae and filled fibres could be seen in the white matter sweeping away from area 21a. This experiment resulted in the labelling of fibres and terminals in several regions of the visual cortex and once again only a few retrogradely labelled cells were found, suggesting that Biocytin is transported anterogradely over longer distances in the white matter. Details of Biocytin labelling are presented in the following analysis.

FIGURE 3.7

A schematic illustration which summarises the connectivity of area 21a with other areas in the ipsilateral cortex based on the results of HRP-WGA transport. The heavy lines indicate the densest projections. The main diagram shows the laminar location of cells in the 11 areas of the cortex which project to area 21a whilst the inset shows the laminar pattern of projections from area 21a to area 17.

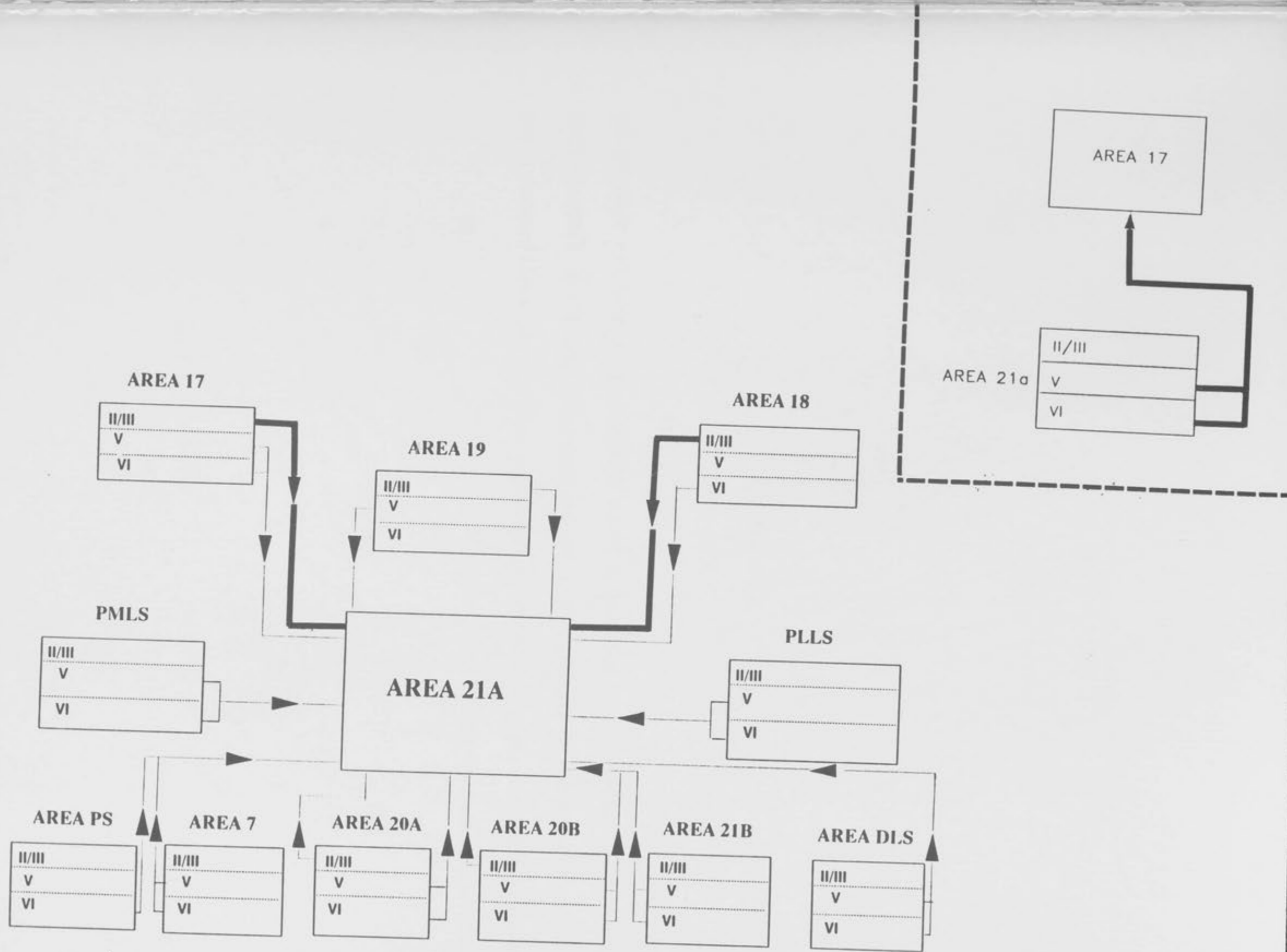


FIGURE 3.8

Drawing tube illustration of a typical Biocytin injection site in area 21a showing the spread of the tracer. Dotted lines indicate the pattern of lamination. Densely filled processes were most apparent in the supragranular laminae and could be seen in the white matter sweeping away from area 21a.



BC1 SLIDE A7

1MM

(1) Areas 17 and 18

The most extensive labelling of terminals and fibres occurred in areas 17 and 18. Figures 3.9a and 3.9b are drawing tube illustrations of typical labelling in the striate cortex which shows that laminae 1, 2/3, 5 and 6 contain the heaviest labelling. The dense concentration of filled fibres in the supragranular and infragranular laminae is especially evident in Fig. 3.9a. An indication of the laminar boundaries is shown in Fig 3.9b.

Figure 3.10 is a photomicrograph of the dorsal edge of area 17 showing densely filled fibres and axon terminals, particularly in laminae 2/3. In area 17 and 18 labelled terminals were present at all laminar levels except lamina 4 which contained traces of labelled fibres only. The terminals in lamina 1 were quite densely filled and fibres were frequently visible for several millimetres parallel to the pial surface or projecting into, and across laminae 2/3. Whilst this can be seen in Fig. 3.10, it is better illustrated in Fig. 3.11 which is a photomicrograph of a section of laminae 1 and 2 of area 17.

Whilst most filled axons were fine, comparatively thick examples, of about $1\mu\text{m}$ diameter, were often present in lamina 1. Lamina 1 was often heavily labelled although it was not possible to determine whether this finding was an artifact associated with darker staining at the edges of sections or whether it was characteristic of axons in this lamina. Figure 3.12a depicts typical biocytin labelling in area 17 with characteristically heavy staining in lamina 1. Throughout the cortex, terminal arbors were often characterised by swellings which indicated presynaptic structures. Figure 3.12b, c and d illustrate three types of varicosities including serial swellings, clusters and single boutons on stalks.

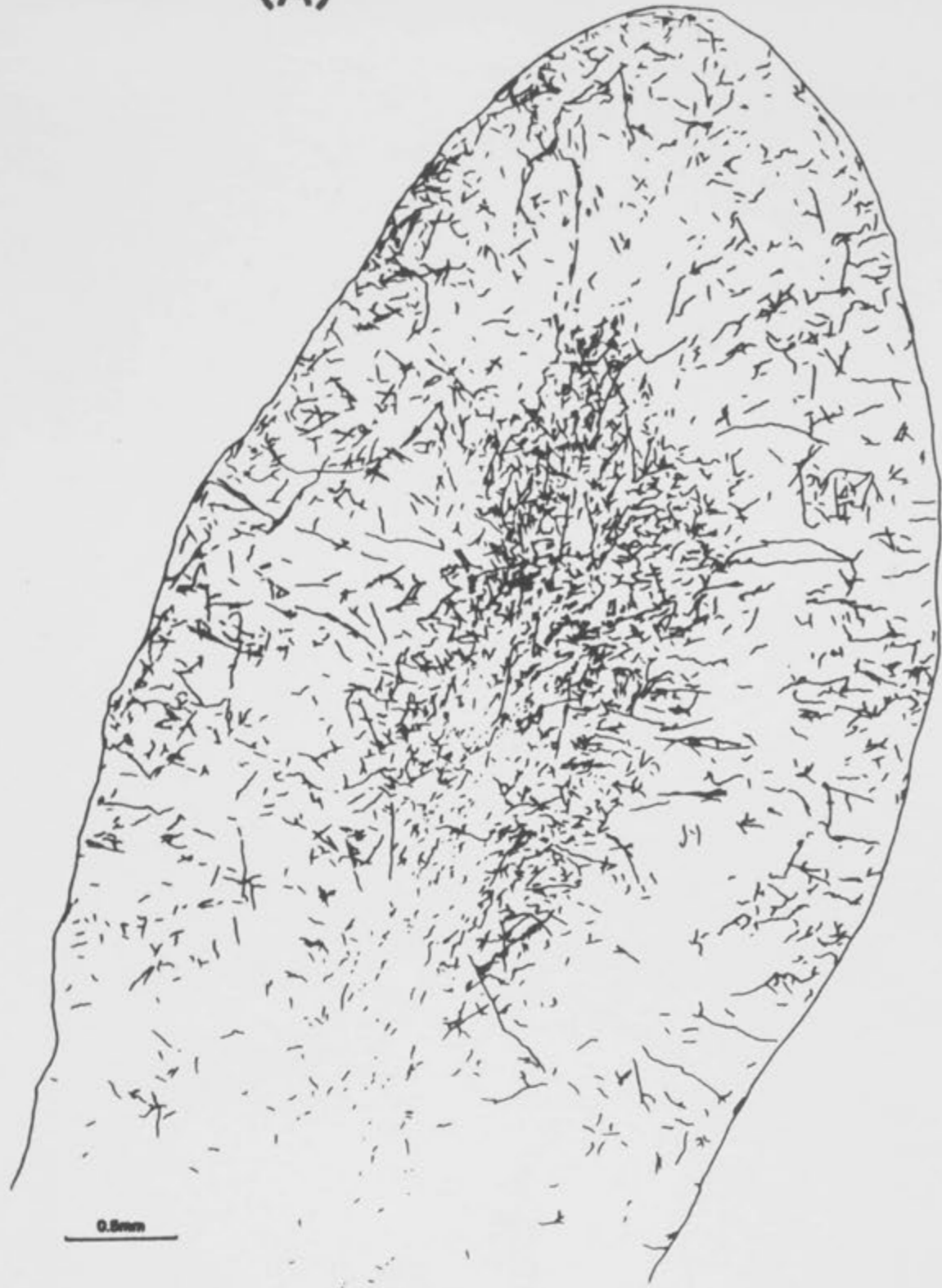
FIGURE 3.9

Drawing tube illustrations of Biocytin labelling in the striate cortex which resulted from a tracer injection into area 21a.

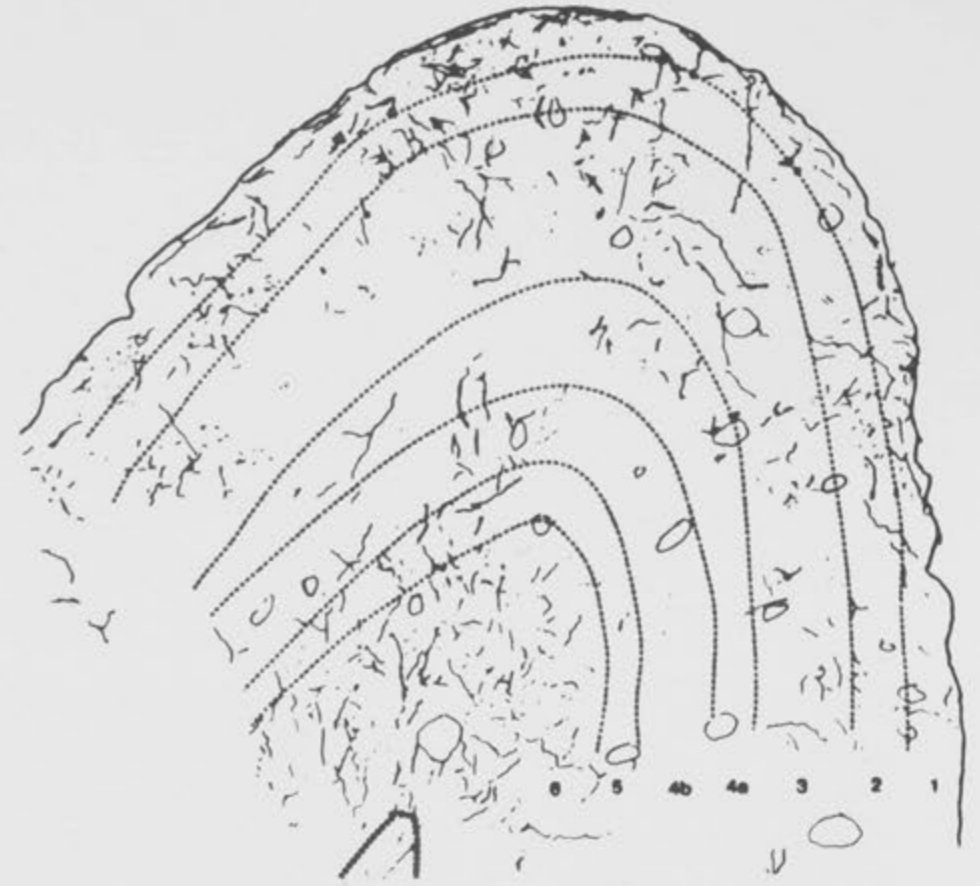
Fig. 3.9(A) is a drawing of a coronal section through the cortex depicting the pattern of labelling in area 17. The heaviest labelling involved fibres and axon terminals in the supragranular and, especially, the infragranular laminae. The granular lamina, however, apart from some filled fibres, was comparatively free of label.

Fig. 3.9(B) is another drawing of labelling in the striate cortex indicating the laminar divisions. Again the heavy labelling in laminae 1, 2, 3 5 and 6 is apparent.

(A)



(B)



BC1 SLIDE C54

1MM

FIGURE 3.10

A photomicrograph of part of a coronal section through the dorsal portion of area 17 showing densely filled fibres and axon terminals which resulted from an injection of Biocytin into area 21a. The illustration covers laminae 1, 2 and 3. Long fibres in lamina 1 often appeared to run parallel to the pial edge of the cortex whilst other extended across laminae 2 and 3. The scale bar = 100 μ m.

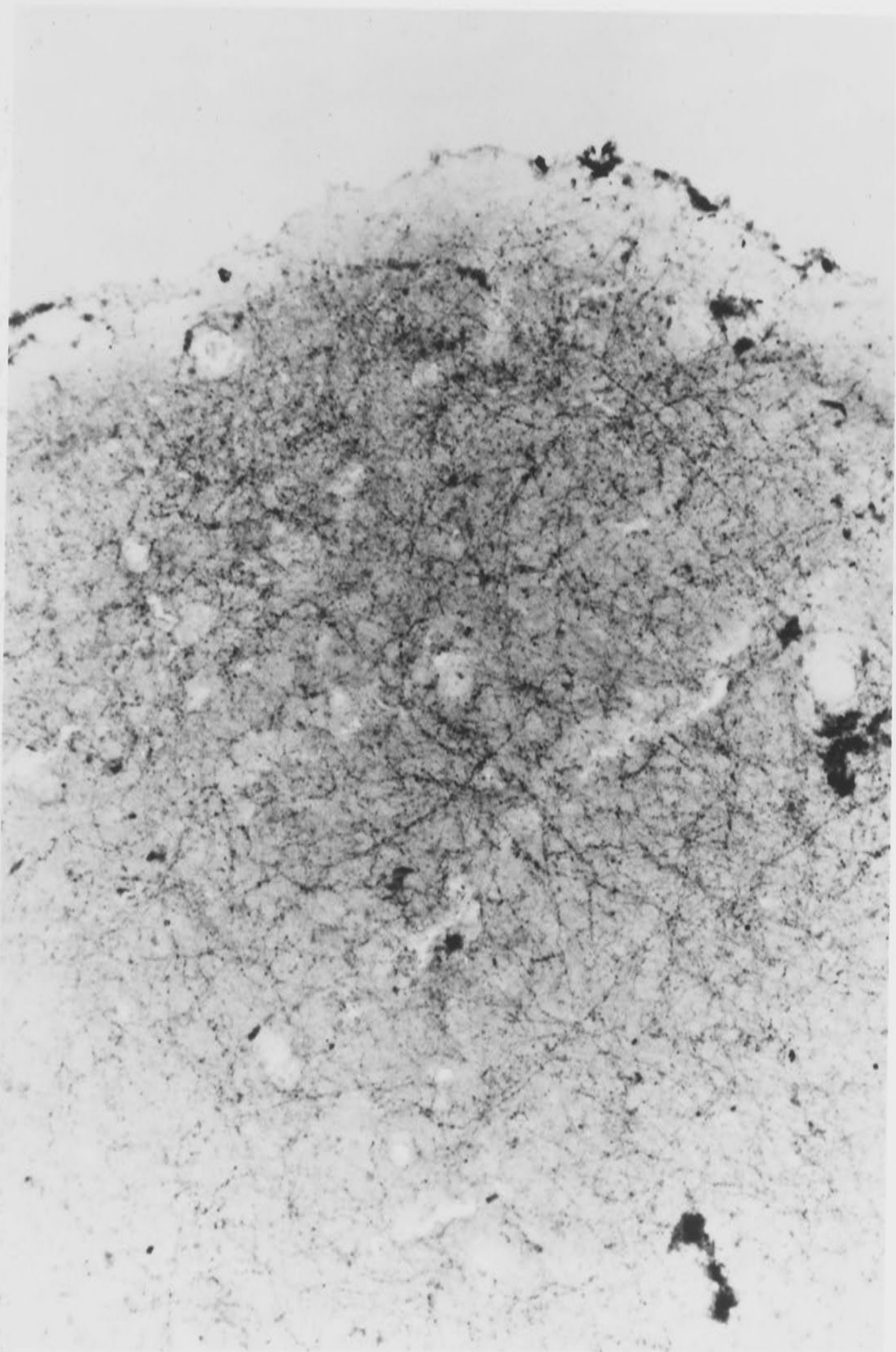
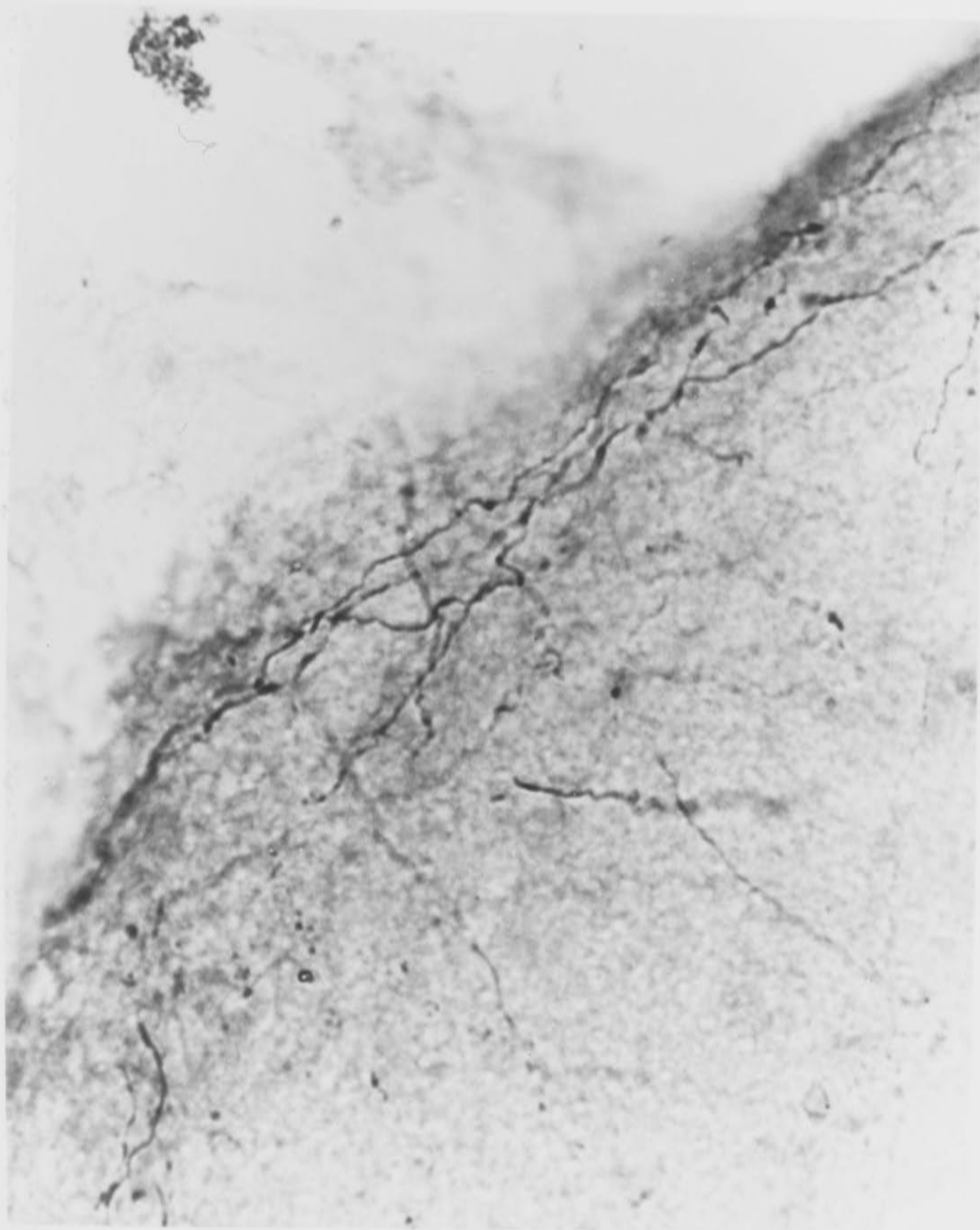


FIGURE 3.11

A second photomicrograph of part of area 17 taken at a higher magnification to show typical Biocytin filled fibres in laminae 1 and 2. The scale bar = $10\mu\text{m}$.



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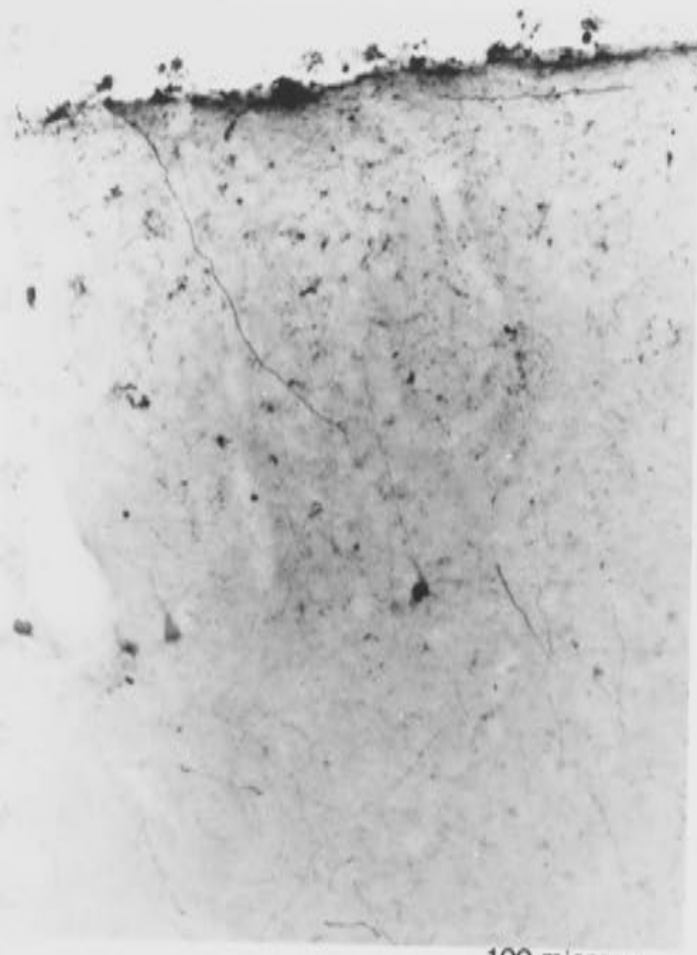
FIGURE 3.12

Photomicrographs of Biocytin labelled fibres and axon terminals in area 17 which followed an injection of the tracer into area 21a.

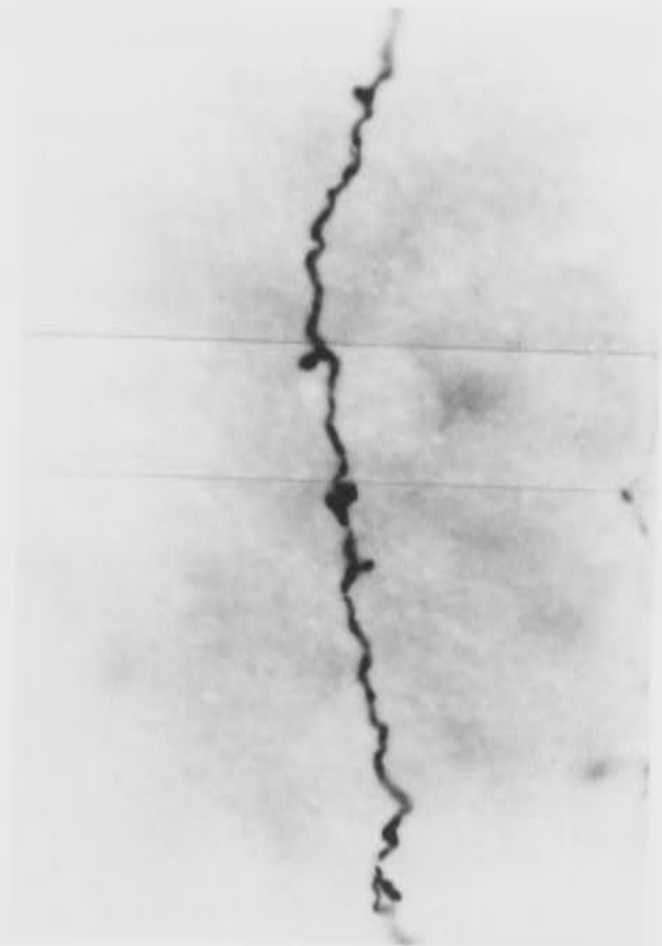
Fig. 3.12(a) is part of a coronal section depicting labelling which occurred in area 17. Once again long axons in lamina 1 which run parallel to the pial edge and others which extend across the cortex perpendicular to the cortical surface, are visible.

Figs. 3.12(b)-3.12(d) are highly magnified enlargements of processes which are present in Fig. 3.12(a) and are typical of Biocytin filled structures found throughout the cortex. Terminal arbors were often characterised by swellings indicative of presynaptic structures. Three types of varicosities shown in these figures are serial swellings, clusters and single boutons on stalks.

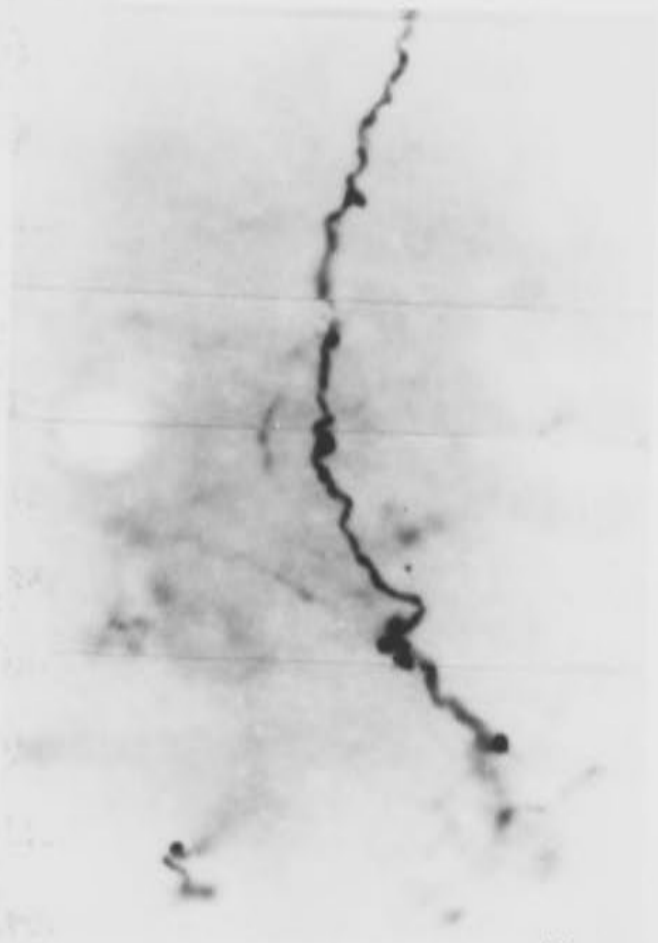
(a)



(b)



(c)



(d)



(2) Other Cortical Areas

Other regions of the visual cortex were generally not as heavily labelled as the primary visual areas and visualisation of the fine axons and boutons often required high magnification (X500 or greater). In areas 19, 20a and 20b filled terminals and fibres were present in laminae 1 and 2/3 but in other laminae labelling could not be discerned with certainty. In the PLLS and DLS, however, lightly labelled terminals and fibres occurred in laminae 1, 2/3, 5 and 6. The most prominent labelling of the association cortex occurred in PMLS, where axon terminals and fibres were present in laminae 1, 2/3, 5 and 6. In no area of the association cortex was there evidence that lamina 4 contained any filled terminals although there were occasional traces of labelled fibres.

Fluorescent Labelling

Overlap of Projections

Injections of fluorescent dyes were made into two separate regions of area 21a (FB injected in caudal location and DY rostral). The main aim of this experiment was to determine whether separate populations of cells in the striate cortex projected to separate locations in area 21a. In the event, filled neurons were visible in areas 17, 18, 19, 20b, PMLS, PLLS and DLS. Despite the fact that lamination of the cortex is not visible under fluorescent luminance, the laminar location of FB and DY labelled cells was judged to be similar to that which resulted from the retrograde transport of HRP-WGA, as described above. Unfortunately, damage to the ventral and lateral edges of a number of sections prevented a thorough investigation of FB and DY labelling in areas 20a and 21b

Figure 3.13 presents photomicrographs taken at a magnification X500 of typical fluorescent labelling in area 17. Although there was some overlap of cell populations, FB and DY labelled cells generally occupied separate AP locations. It was apparent in the striate cortex that the main population of FB labelled cells was situated more caudally than the major population of DY labelled cells. The FB labelled cell population was first visible in sections located at HC: -9.0mm AP and it became progressively more dense anteriorly, reaching its heaviest at HC: -7.0mm AP. A typical example of two FB labelled cells which were located at about HC: -7.0mm AP is depicted in Fig. 3.13(A). Whilst an occasional DY labelled cell was detected in sections as posterior as HC: -8.0mm AP, the heaviest labelling was visible in sections at HC: -4.0mm AP. A cluster of DY labelled nuclei located at HC: -4.0mm anteroposterior is presented in Fig. 3.13(B). An example where the two cell populations overlapped is depicted in Fig. 3.13(C) which shows two cell nuclei labelled with DY and one cell filled with FB. However, there was no conclusive sign of any double labelled cells.

Overview

The results of this study demonstrated that area 21a has strong reciprocal connections with a number of areas of the primary and association visual cortex most of which contain retinotopic representations of the central part of the visual field.

The strongest connections appeared to be with areas 17, 18 and, to a lesser extent, 19. Neurons in the supragranular laminae of areas 17 and 18 provided the densest efferents to area 21a whilst considerably less dense projections emanated from cells in the infragranular laminae, especially lamina 6. Pathways from separate regions of

FIGURE 3.13

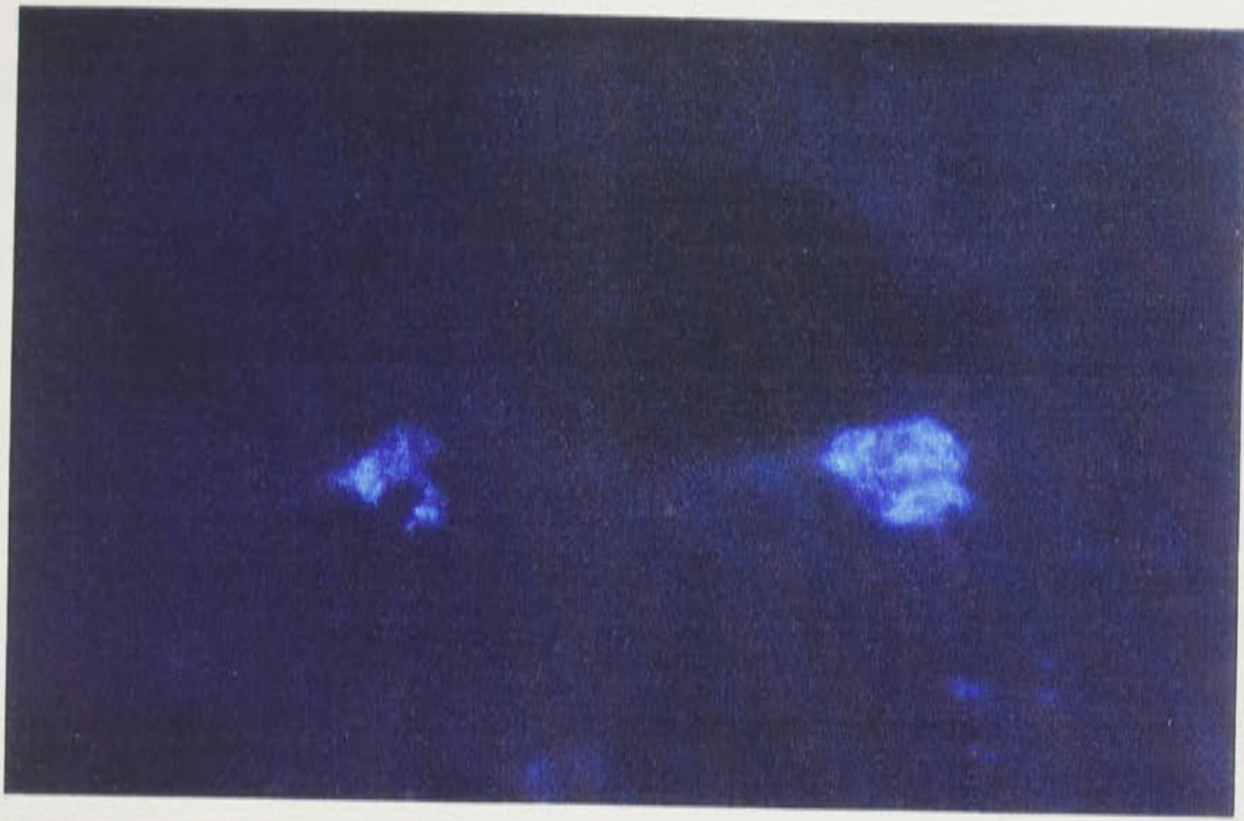
Photomicrographs of fluorescent labelled cells in area 17 which resulted from paired injections of Diamadino yellow (DY) and Fast blue into area 21a. Two largely separated populations of cells were detected and there was no indication that any were double labelled. The scale bar = 50 μ m.

Fig. 3.13(A) shows two cells labelled with FB. These were located at HC -7.0mm AP.

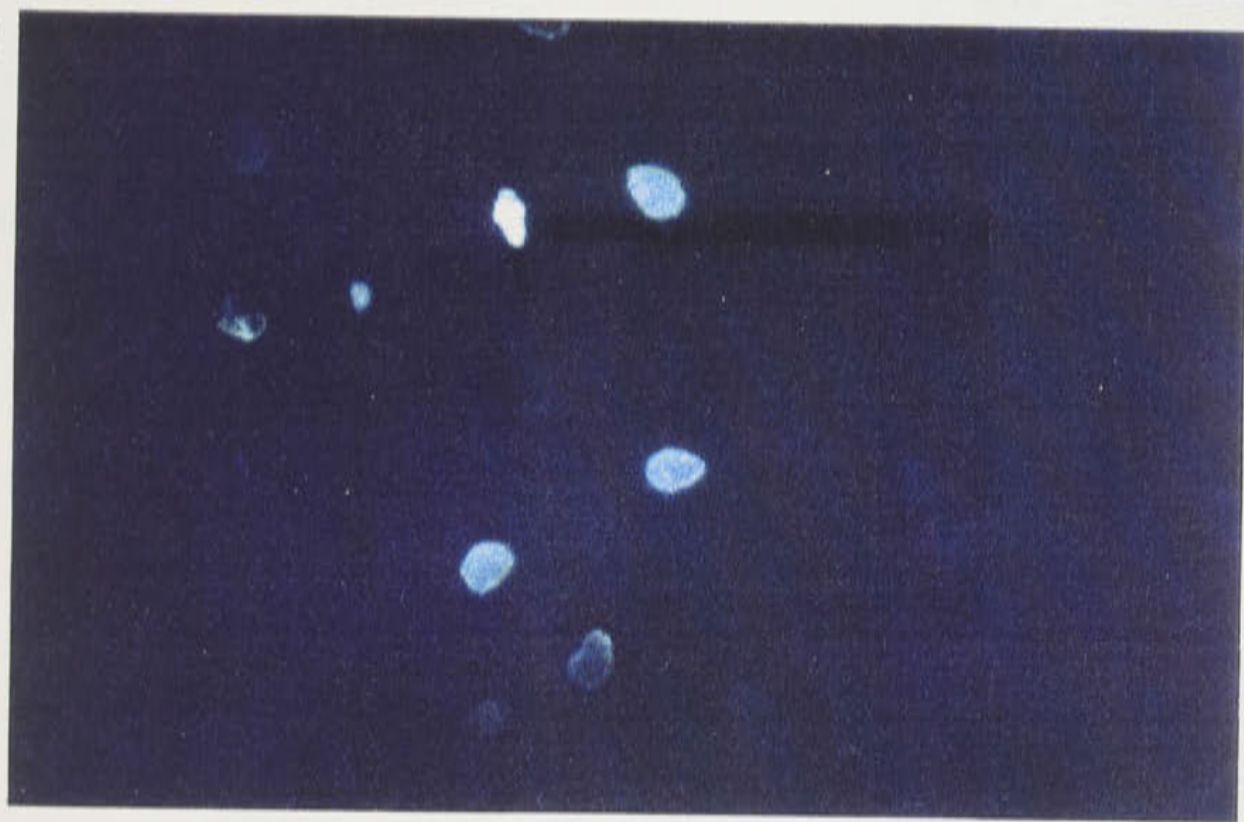
Fig. 3.13(B) illustrates a cluster of DY labelled cells which were located at HC -4.0mm AP.

Fig. 3.13(C) shows the nuclei of two cells filled with DY and one cell labelled with FB.

(A)



(B)



(C)



area 17 innervate separate locations of area 21a. Projections to area 21a from area 19 appeared to originate from the supragranular and infragranular laminae in roughly equal proportions.

The situation with respect to afferent projections from areas of the association cortex is apparently quite different. Labelling was generally not as heavy as occurred in the primary visual cortex and in all cases the densest inputs to area 21a arose from cells located in the infragranular laminae. In only two cases could projections from the supragranular laminae be detected and both were very sparse.

Efferent projections from area 21a terminated in the supragranular and infragranular laminae of a number of areas in the primary and association cortex. Although filled fibres were present in lamina 4, there was little evidence of labelled boutons.

DISCUSSION

Relative Strength of Cortical Projections to Area 21a

Since area 21a contains a retinotopic representation of the central portion of the visual hemifield (Tusa and Palmer, 1980), it was anticipated that it would connect only with areas of the cortex involved with analysing the central part of the visual percept. The results provide general confirmation of this expectation. Most connections are either with areas whose function is entirely devoted to analysing a representation of the central part of the visual hemifield or with areas, where the amount of cortex allocated to representing the central portion of the visual hemifield, is proportionally substantially greater than that devoted to the representation of the peripheral part of the visual hemifield. These regions are reported to comprise areas 17, 18, 19, 20b, 21b, PMLS, PLLS and DLS (Dreher, 1986). More specifically, the results provide convincing evidence that the most abundant, and presumably the most influential, projections to area 21a arise from neurons located in areas 17, 18 and to a lesser extent 19. By contrast, projections from areas 20b, 21b, PMLS, PLLS and DLS appeared to be considerably weaker.

In addition to these pathways, a weak connection appears to exist between area 21a and area PS. This specific pathway has not been reported previously although Sherk (1986) has spoken of the existence of a relatively weak projection from PS to area 21 as a whole. A connection between PS and area 21a is not inconsistent with neuronal circuitry concerned with processing images which are centrally located in the visual field. The PS region is visually responsive and contains a retinotopic representation covering an elevation of $+5^{\circ}$ to -30° and an azimuth from the vertical meridian to

25° (Updyke cf Rosenquist, 1985). Although these co-ordinates cover a large extent of visual space they do encompass a significant part of the central visual field represented in area 21a.

In contrast to circuitry involving regions which specialise in central vision, my results indicated there is a connection with area 20a in which the peripheral portion of the visual hemifield is emphasised (Dreher, 1986). This connection has also been reported by Symonds and Rosenquist (1984a). Although the purpose of the connection is not known, it possibly acts to integrate data from the central representation of area 21a with the overall processing taking place in area 20a. The retinotopic representation in area 20a is reported to contain the central 50° of the upper visual field and perhaps 20° of the lower field which extends out to 90° along the horizontal meridian (Tusa and Palmer, 1980). The extent of this coverage incorporates the retinotopic representation contained within area 21a.

The connection between area 21a and area 7 also has been found by Symonds and Rosenquist (1984a). Area 7, part of the parietal region of the cat cortex which lies along the crown of the middle suprasylvian gyrus, has been divided into several areas on the basis of cytoarchitectural, connectional and physiological investigations (see Rosenquist, 1985). Physiological evidence indicates that area 7 is a multifunctional region where visual, somatic and auditory modalities converge. Despite the fact that it lacks any retinotopic organisation, the existence of connections with cortical and subcortical visual areas suggests that area 7 should be classed as a visual region (Rosenquist, 1985).

The relative strength of corticocortical connections is almost certainly indicative of their importance and perhaps the type of information they convey. In the present study area 21a appears to have major connections with areas 17 and 18 which suggest

that the responsiveness of cells in area 21a will be heavily dependent on the inputs from the primary visual cortex. By contrast, since the connections with area 19 and regions of the association cortex were generally weaker, it would seem that those areas have considerably less influence on the responsiveness of cells in area 21a.

The presence of patches or clusters of HRP-labelled cells and terminals in areas 17 and 18 might well reflect functional aspects of the neurons projecting to area 21a. These discontinuities in labelling suggest that the primary visual cortex is connected to area 21a by fascicle-like bundles of axons. The function of these bundles might be to conduct visual signals that are qualitatively different from one another and which terminate in discrete regions in area 21a. In the past, there have been numerous reports that intercortical connections originate from discontinuous patches (Jones et al., 1975; Kunzle, 1976; Goldman and Nauta, 1977; Imig and Brugge, 1978; Wong-Riley, 1979; Gilbert and Wiesel, 1980, 1981; Montero, 1980; Tigges et al., 1981; Bullier et al., 1984b; Symonds and Rosenquist, 1984a). Anatomical periodicities in corticocortical connections in the cat were reported originally for areas 17, 18, 19 and PMLS (Gilbert and Wiesel, 1980, 1981; Montero, 1981), but Symonds and Rosenquist (1984a) observed patches of label in 13 additional cortical areas including area 21a. The significance of these patches, especially the way in which they correlate with underlying function, remains a subject for much speculation. It is significant in the present context that the patches do not persist in the feedback pathways.

Whilst several features of visual encoding such as ocular dominance, orientation specificity and direction selectivity are known to be distributed throughout the cortex in periodic bands, many early experiments failed to demonstrate that they are related to the discontinuities in projection patches. In fact Gilbert and Wiesel (1981) concluded that functional characteristics such as ocular dominance and orientation

were not associated with corticocortical projection patches. Despite their conclusion, the possibility that functional aspects of visual perception are transmitted between areas along discrete streams could not be ruled-out and Gilbert and Wiesel (1989) combined 2-deoxyglucose autoradiography with HRP tracing to demonstrate that patches of HRP labelling in areas 17 and 18 are, in fact, related to orientation columns.

The neurons of area 21a are distinguished by their acute orientation tuning (see Chapter 3) and appear to receive their major inputs from the striate cortex (see also Chapter 5). Likewise, many cells in the striate cortex are distinguished by their acute orientation tuning (Henry et al., 1973; Henry et al. 1974a,b). If, as suggested in Chapters 3 and 5, orientation tuning in area 21a is determined by neurons in the striate cortex, axons which convey orientation specific signals between these two areas might follow separate streams.

The existence of two largely separate populations of fluorescent-labelled cells in area 17 demonstrated that the spatial separation of the processes in area 17 is maintained in area 21a. Failure to find any double labelled cells among these populations suggested that there is little overlap of their terminal arborisations in area 21a. These results were consistent with the proposition that the segregation of pathways which was manifested in patches in area 17 could persist in area 21a. Patchiness, however, has not been reported in any projection arising from area 21a.

Reciprocity of Corticocortical Connections

Reciprocity of corticocortical connections was indicated by the presence of mixed populations of labelled cell bodies and terminals in a number of cortical areas which

resulted from the transport of HRP-WGA from area 21a. The existence of reciprocal connections between areas 17, 18, 19 and the lateral suprasylvian areas has been demonstrated by Garey et al. (1968); Wilson (1968), Kawamura (1973) and Bullier et al. (1984b). In the present study, the finding that the connections between area 21a and areas 7, 17, 18, 19, 20a, 20b, 21b, DLS, PMLS, PLLS and PS were bi-directional is in keeping with the conclusion of Symonds and Rosenquist (1984a) who reported that reciprocity is a feature of all corticocortical connections.

Little is known of the properties and purpose of these reciprocal pathways but possibly they act to modify incoming data such as noise suppression in the input or for interaction with data from other areas. Rosenquist (1985) is of the opinion that the existence of widespread reciprocity of connections diminishes the likelihood that cortical areas act within a straightforward serial or hierarchical sequence.

Laminar Circuitry

Background

A number of attempts have been made, especially in the primate, to arrange cortical areas into a hierarchical scheme based on connections between cortical laminae (Jones and Wise, 1977; Kaas et al., 1977; Tigges et al., 1977, 1981; Rockland and Pandya, 1979; Weller and Kaas, 1981; Maunsell and Van Essen, 1983; Van Essen, (1985). For example, the majority of rostrally directed projections in primates arise mainly in lamina 3 and terminate in and around layer 4 of adjacent areas while caudally directed reciprocal projections originate mainly from cells in laminae 5, 6 and to a lesser extent in 3a and terminate largely in lamina 1 (Pandya & Yeterian, 1985).

Gilbert and Kelly (1975) considered that in the cat cortico-cortical connections arise from neurons in either laminae 2/3 or lamina 6. Bullier et al. (1984b), on the other hand, take the view that there are always cortico-cortical neurons in laminae 2/3, 5 and 6, and that the proportion varies depending on the areas involved in the connection. Their findings lead them to propose that the laminar distribution of corticocortical neurons depend on the area of origin rather than on the target area. For example, according to their model corticocortical cells in areas 17 and 18, which are the main geniculorecipient areas, are located mainly in laminae 2/3 whilst those of the distantly placed area 20a arise predominantly from laminae 5 and 6. Areas such as 19 and PMLS which are located between these extremes have similar proportions of afferent cells in both the supragranular and infragranular laminae. Results of the current experiments in which differences existed in the laminar distribution of projections to area 21a from the primary visual cortex (areas 17 and 18), area 19 and several regions of the association cortex are not inconsistent with their conclusion.

Henry (1991) has pointed out that ambiguous results in projections linking various parts of the cortex lead Symonds and Rosenquist (1984a) to propose that circuits from different extrinsic regions pass through different laminae to allow the area to contribute to a number of cortical loops. They suggest that, since each cortical area has multiple inputs and outputs and the functions of any one area depend upon on how it participates in the various circuits of which it is a part, attention should be directed to circuits rather than to specific areas.

Evidence from tracer experiments alone, such as those undertaken in the present study, is unlikely to define the purpose of these multiple loops. Nevertheless, the pattern of connectivity demonstrated for area 21a is in keeping with a complex model

of information flows whereby visual data might be continuously relayed backward and forward between area 21a and numerous cortical visual areas. An information processing model in which separate circuits are based on specific laminar connectivity arising in particular areas would allow for greater functional specialisation (as suggested by Bullier et al., 1984b) and expedite the simultaneous modification, integration and processing of signals. A tentative hypothesis derived from Symonds' and Rosenquist's (1984a) proposal is that each connecting loop carries visual data which is qualitatively different as a result of modification performed in the specific lamina through which it passes.

There has been speculation that projections arising in supragranular laminae (termed *feedforward* projections) provide essential visual information, whilst those arising in infragranular laminae (*feedback* projections) provide modulating influences (Lin et al., 1982; Orban, 1984; Rockland and Pandya, 1979; Tigges et al., 1981). With respect to the cat, Symonds and Rosenquist (1984b) attempted to construct a hierarchy of visual areas based on percentages of *feedforward* and *feedback* pathways but they were unable to achieve an unambiguous hierarchical scheme for all the areas investigated (Rosenquist, 1985). In the present study the relative strengths of feedforward and feedback pathways are used to allocate a position to area 21a in the visual hierarchy. Details of these pathways are analysed in the following discussion.

Laminar Connectivity with Areas 17, 18 and 19

Results from the present experiments indicate that projections to area 21a arise from cells in the supragranular and infragranular laminae of areas 17, 18 and 19. In the case of areas 17 and 18 projections which originate from the supragranular laminae are the major ones (more than 80% of the projecting cells in area 17). This contrasts with area 19 where projecting cells are evenly distributed between the supragranular

and infragranular laminae. In their study of laminar connections in cat visual cortex, Symonds and Rosenquist (1984b) did not report projections to area 21a from the infragranular laminae of areas 17, 18 and 19 although Lee et al., (1982) noted that more than 80% of cells projecting to area 21a from these areas were located in laminae 2 and 3 whilst the remainder came from laminae 4, 5 and 6. In findings similar to those of the present study, Bullier et al., (1984b) reported that corticocortical neurons in the main geniculorecipient areas 17 and 18 belong mainly to laminae 2 and 3 whilst area 19 contains similar proportions of corticocortical neurons in both the infragranular and supragranular laminae.

It appears, therefore, that the projections to area 21a from neurons in the supragranular laminae of areas 17 and 18 represent major *feedforward* pathways for transmitting information from the LGNd. Area 19 may also contribute to this transfer of signals but it is not one-sided in feeding information from the supragranular laminae. The presence of equal though somewhat weaker projections to area 21a from both the supragranular and infragranular laminae of area 19 indicates that area 19 holds a different position in the processing sequence vis a vis area 21a than is held by areas 17 and 18. The finding also suggests that areas 19 and 21a might be engaged in an equal exchange of both *feedforward* and *feedback* information.

The differences that were apparent in the relative strengths of the projections from the supragranular and infragranular laminae of areas 17 and 18 when compared to those of area 19 might reflect differences in the origin of inputs from the LGNd. For instance, areas 17 and 18 are the only cortical regions to receive afferents from layers A and A1 of the LGNd. Bullier et al. (1984a) point-out that both areas receive similar and often common subcortical inputs and are interconnected by cells occupying similar laminar positions in each area (Bullier et al., 1984b). The views of

Bullier et al., together with my own findings that the major and minor projections to area 21a arise from identical laminae in areas 17 and 18 are consistent with the proposition that these two areas function in parallel (Tretter et al., 1975; Orban, 1984). The results suggest that areas 17 and 18 occupy a similar hierarchical level in the processing of visual information and their role is probably analogous to that of area V1 (area 17) in the monkey (Bullier et al., 1984b). However, since only area 17 receives direct input from geniculate X-type cells while area 18 receives its principal geniculate input from Y-type cells, it cannot be assumed that the information each area supplies to area 21a is of an identical nature.

Unlike areas 17 and 18, area 19 is not innervated from the A layers of the LGNd; its main thalamic input comes from the C layers which contain mainly W-type cells (Dreher, 1986). Area 19 is likely, therefore, to be functionally quite distinct from the primary visual cortex (areas 17 and 18) and this distinction may be reflected in the different ratio of the infragranular and supragranular contributions to area 21a.

The absence of large numbers of retrogradely labelled cells in lamina 4 of any area of the visual cortex following the injection of areas 21a and 17 with axonal tracers is consistent with the proposition that, in the cat, this lamina is concerned primarily with receiving and integrating sensory inputs from sub-cortical regions. On the other arm of the reciprocal pathway lamina 4 is not heavily implicated in corticocortical connectivity as revealed from the use of Biocytin as an anterograde tracer in area 21a. Thus the Biocytin investigation revealed that throughout the cortex lamina 4 appeared to be free of labelled terminals although traces of fibres could be detected.

The proposition that lamina 4 is concerned mainly with receiving sub-cortical signals appears to hold for connections with the lateral posterior nucleus (LPI) where the

injection of HRP-WGA resulted in terminal labelling in lamina 4 (see Chapter 1). In their study of the laminar distribution and branching pattern of corticocortical neurons, Bullier et al. (1984b) reported that in all cortical areas examined there was a region deep in the cortex which was largely devoid of corticocortical neurons. They considered that this region corresponded to the major part of lamina 4 in areas 17 and 18, to lamina 4 and the lower portion of lamina 3 in areas 19 and PMLS and to laminae 2, 3 and 4 in area 20. They were not able to determine, however, whether this region of lamina 4 corresponded to the zone of thalamo-cortical axonal termination. Symonds and Rosenquist (1984b) also reported a minor projection from lamina 4 of area 19 to area 21a. The conclusion that lamina 4 in the cat plays little role in intercortical connections stands in contrast with the pattern of cortical connectivity in primates in which rostrally directed projections arise at the supragranular level and terminate in or around lamina 4.

In summary, there is justification in proposing that *ascending* or *feedforward* pathways to area 21a arise principally from areas 17 and 18, with a lesser component from area 19. Since the geniculo-cortical pathways arising from the A layers terminate in areas 17 and 18, it is reasonable to assume that the *feedforward* pathways from these areas conduct the bulk of visual input to area 21a. The relative differences in the proportion of laminar projections arising from the primary visual cortex compared to those from area 19 suggest that each might lie at a different level in the hierarchy of visual processing.

Laminar Connectivity with the Association Cortex

The pattern of laminar connectivity between area 21a and several regions of the association cortex is quite dissimilar to the pattern of connections to area 21a which originates in the primary visual cortex and area 19. In all association areas where

labelling was detected, the highest proportion of cells contributing to projections to area 21a comes from neurons in the infragranular laminae. In two areas where labelled cells were found in the supragranular laminae (areas 20a and 20b) the projections are comparatively weak. The purpose of pathways to area 21a which arise from areas of the association cortex remains unresolved but their origin in the infragranular laminae raises the possibility of their *feedback* role in order to modify area 21a's specific processing functions.

Duplication of Projections

The duplication of inter-cortical pathways from cells in the supragranular and infragranular laminae of the same area was a feature of projections to area 21a from areas 17, 18, 19, 20a and 20b. Although not a new finding, the reason for twin pathways is unclear. Duplication of laminar connections is a common occurrence throughout the visual cortex but it seems very unlikely that the purpose of a second pathway is to provide redundant information. If it were, then one would expect that connections which carried identical signals, but which arose from two different laminar levels, would be of similar density. Yet only in the case of area 19's projections to area 21a were efferents from the supragranular and infragranular laminae of approximately equal strength. Apart from confirming the presence of duplicate projections, the present study adds little to a knowledge of their function.

Overview

The pattern of corticocortical connections resulting from this investigation indicates that area 21a receives its main inputs from the primary visual cortex, particularly area 17 where major pathways arise from the supragranular laminae (*feedforward*

pathway). In area 19, somewhat weaker projections arise from both the supragranular and infragranular laminae in approximately equal strength which indicates, perhaps, that areas 19 and 21a might engage in an equal exchange of *feedforward* and *feedback* data. Differences in the laminar distribution of cells projecting from the primary visual cortex compared to those from area 19 provide a strong indication that each lies at a different hierarchical level in the sequence of visual processing.

Projections to area 21a also arise from several regions of the association cortex. These afferents are generally weak and arise primarily from the infragranular laminae (*feedback* pathway). All corticocortical connections are reciprocal although the reasons for this are not known.

Thus the anatomy suggests that there is a multiplicity of inputs to area 21a but most of them are *feedback* pathways and it will be of great interest to discover how much these return inputs influence the development of a serial sequence of information processing.

Proposed Model of Information Flow

The present tracer results provide a comprehensive picture of the laminar location both of cells projecting to area 21a and of the termination of projections from cells in area 21a. As such, the findings form a good basis upon which to construct a model of cortical information flows.

The terms "feedforward" and "feedback" are restrictive, in that they imply a functional role which has become rigidly related to the supra- and infragranular laminae of any cortical region. That is to say, cells of the supragranular laminae are always regarded as contributing to a feedforward pathway whilst those in the infragranular laminae are supplying a feedback pathway. Whilst there is much evidence to support this relationship the results suggest that such an interpretation only holds true if two areas are in sequential association. In such a relationship the feedforward pathway from the supragranular laminae seems to be equivalent to the feedback pathway from the infragranular laminae. In other words the contribution from the supragranular laminae to the path going one way is equal to that from the infragranular laminae to the path going the other way. This rule holds for both directions. This equation, however, breaks down for two areas which act in parallel, where interconnections may be more involved in cross-referencing that in advancing or modulating the processing sequence. This interpretation appears to be consistent with many of the intercortical relationships examined in the cat (see Bullier et al., 1984b).

For the present needs - that of deciding the extent to which area 21a is sequentially supplied by areas 17, 18 and 19 - it seems worthwhile to turn the reasoning around and to use the degree to which the equation is obeyed as a pointer to the level of sequential relationship between pairs of areas. To put the requirements for a sequential relationship in semi-mathematical terms, therefore, and using the paths linking areas 17 and 21a as an example, results in the following relationship:

$$(P_{s17} + P_{s21a} = P_{i17} + P_{i21a})$$

where P_{s17} and P_{i17} represent projections from the supragranular and infragranular laminae of area 17, respectively. P_{s21a} and P_{i21a} represent similar relationships in respect of area 21a. The stronger of the two projections from the supragranular laminae (in this example, from area 17) indicates the direction of progression for

processing and their relative strengths should be mirrored by those of the projections from the infragranular laminae.

The testing of this relationship for a variety of areas suggests that the links of area 21a with areas 17, 18 and 19 are essentially sequential (i.e. the equation holds). In these examples the forward flow from areas 17 and 18 is predominantly in one direction whilst from area 19 it is about equal in both directions. By contrast, an example of non-serial flow is to be found in the relationship between areas 17 and 18 where the supragranular projections in both directions completely outweigh the infragranular projections (Bullier et al., 1984b). In terms of the relationship:

$$P_{s17} + P_{s18} > P_{i17} + P_{i18}$$

In this case the equation for serial processing breaks down and the two areas act in parallel with the links between them acting to cross reference rather than to advance or modulate the processing of information. The case for serial processing cannot be sustained either in connectivities between areas 17 and 18, on the one hand and area 19, on the other. According to Bullier et al., (1984b) there are strong projections from the supragranular laminae of areas 17 and 18 to area 19 and weak ones from the infragranular laminae. In return, area 19 projects strongly to areas 17 and 18 from both the supragranular and infragranular laminae. In terms of the relationship:

$$P_{s17} + P_{s19} > P_{i17} + P_{i19}; \text{ and}$$

$$P_{s18} + P_{s19} > P_{i18} + P_{i19}.$$

Support for this model could come from further experiments which investigate the laminar distribution of afferent cells in all areas of the visual association cortex. With respect to area 21a, additional research into the laminar location of the area's projections to higher cortical areas is planned.

Conclusion

The major conclusion to be drawn from this study is that it provides anatomical support for the notion that area 21a is serially linked with areas 17 and 18 from which it appears to receive its major inputs. Both these areas seem to lie at the same hierarchical level. This notion will be further acknowledged in later chapters. Some lesser cortical input may also arise from area 19.

CHAPTER 4

RESPONSE CHARACTERISTICS OF THE CELLS OF AREA 21A

Pattern detection is the basis of vision and involves responses to a wide range of spatial frequencies. If the function of the primary visual cortex were to improve pattern resolution, it follows logically that removal of the primary visual cortex should lead to pattern blindness. It has already been pointed out that in a number of species including man, apes, rabbits, tree shrews and cats this is not the case.

The ability of an animal to discriminate patterns following removal of the primary visual cortex may be studied in a number of possibilities. For example, re-organization of the remaining fields of cells to remaining visual areas might result in their assuming the receptive field properties of cells in the primary visual cortex. However, it only is clear in mammals that such a reorganization occurs as the spatial visual areas after cortical ablation (see Darian-Smith and Quinlan (1974) and Spatz and Barman (1976) have shown that some of the normal receptive properties of mammalian visual cortex are not followed by removal of area 17).

INTRODUCTION

Electrophysiological research in the visual cortex of the cat has concentrated predominantly on the contribution that the primary visual cortex (areas 17 and 18) makes to perception. Perhaps this has been due partly to a belief, following the early investigations of Hubel and Wiesel (1959;1962), that contour constituents such as straight line segments, edges, corners, orientation and spatial frequencies were extracted by neurons essentially in these areas. Moreover, being the only cortical areas to receive inputs from the major laminae of the LGNd, together with their relatively easy accessibility for electrophysiological recording, made areas 17 and 18 obvious choices of early experimenters.

Pattern detection is the basis of vision and involves responses to a wide range of spatial frequencies. If the function of the primary visual cortex were to perceive pattern constituents, it follows logically that removal of the primary visual areas should lead to pattern blindness. It has already been pointed-out that in a number of species including rats, squirrels, rabbits, tree shrews and cats this is not the case.

The ability of an animal to discriminate patterns following removal of the primary visual cortex may be attributable to a number of possibilities. For example, reorganisation of the receptive fields of cells in remaining visual areas might result in them assuming the receptive field properties of cells in the primary visual cortex. However, not only is there no evidence that such a reorganisation occurs in the spared visual areas after cortical ablation but Donaldson and Nash (1975) and Spear and Baumann (1979) have shown that some of the normal response properties of extrastriate areas may even be lost following lesioning of area 17.

Another possibility is that animals with an ablated primary visual cortex may develop new perceptual strategies for sampling visual information, although once again there does not appear to be much evidence to support this proposition.

Berlucchi and Sprague (1981) considered it more likely that areas crucial for discrimination of simple patterns or forms almost certainly lay outside areas 17 and 18, in those zones of the suprasylvian regions which are the recipients of major extrageniculate tecto-thalamo-cortical projections. If their hypothesis is correct, the X-type ganglion cells cannot be implicated for whilst these cells possess high spatial resolution which seem to be necessary for acute pattern discrimination, their signals are transmitted through the primary visual cortex. It follows, therefore, that form recognition which occurs in cortical regions outside areas 17 and 18, must be mediated by W- and/or Y-type cells and probably involves the perception of comparatively coarse patterns. The suprasylvian regions which Berlucchi and Sprague believed were most likely involved in some integrated form of perception and discrimination are areas 19, 20, 21 and the lateral suprasylvian zone. It is thought that area 21a in particular, in addition to the important role it almost certainly plays in central vision (Tusa and Palmer, 1980) possibly has a vital function in pattern learning and retention, as well as discrimination (Spear and Braun, 1969; Doty, 1971; Sprague et al., 1977; Campbell, 1978; Berlucchi and Sprague, 1981).

Despite the potential of area 21a in pattern recognition, it has been subject to little detailed physiological investigation. Ho et al. (1982) concluded, on the basis of extracellular recording, that cells of area 21a had larger receptive fields than those of areas 17, 18 and 19 recorded at the corresponding eccentricities. In addition, the cells responded binocularly, preferred slowly moving (cut-off velocity 10^0 /sec or less) stimuli and were orientation selective. More recently,

Mizobe et al. (1988) concluded, also on the basis of extracellular recording, that most cells of area 21a were sharply tuned for stimulus orientation but not for direction selectivity. Herdman et al. (1989) used the C-deoxyglucose technique to demonstrate that area 21a, *inter alia*, may play a part in the cortical modulation of horizontal optokinetic nystagmus.

Building on these earlier investigations, the present inquiry into the response characteristics of area 21a neurons also considered tuning for stimulus orientation but, in order to discover how the interplay of excitation and inhibition contribute to the shape of the orientation tuning curve, the investigation was designed to encompass the organisation of the cells' receptive fields. To assist with this interpretation, tuning curves were prepared for stimuli of different length and a search was made for evidence of flanking inhibitory regions both directly and from the sharpening of orientation tuning as is thought to occur in S or simple cells in the striate cortex. Since flanking inhibition is not always obvious against a background of spontaneous discharge, maximum background activity was induced using a conditioning stimulus.. The presence of inhibition that was manifested in a preference for end stopped stimuli was also sought by examining the length summation curves beyond the excitatory region of the receptive field. The comparison with receptive fields of neurons of the striate cortex also extended to quantitative analysis of the cells' response to moving contours of each contrast polarity and to their dependence on the velocity of movement. To complete the comparison with striate neurons, an examination of receptive field responses to flashing stationary stimuli was undertaken.

On the basis of material gathered in these experiments it was possible to explore firstly the proposition that the cells of area 21a belonged to more than a single functional group and then the degree to which they differed in their response

patterns from the various cell types in the striate cortex. In addition, the results of the present study assisted in postulating a role for area 21a in visual perception.

Animal Preparation

Experiments were performed on 15 adult cats, each weighing between 2 and 3 kg. Each cat was anaesthetized for surgery with intramuscular injections of ketamine hydrochloride (Ketalar, Parke-Davis, 20 mg/kg) plus 0.5 cc of the muscle relaxant, gallamine (Rogipon, Bayer AG, Ltd.). The respiratory rate was controlled, a tracheotomy performed and a cannula inserted. Eye movements were reduced to a minimum by myopic paralysis of the ciliary muscles with bilateral cervical sympathectomy (Mollnes et al. 1967; Kinsman et al. 1969). The animal was then placed in a stereotaxic instrument, and a stainless steel screw which served as an EEG electrode was embedded in the skull above the frontal lobe. A craniotomy was made at Horsley-Clark (HC) co-ordinates 0 to -5.0 mm anterior posterior, and ± 6.0 to ± 5.0 mm medial-lateral, the dura reflected, and a circular well was prepared with dental acrylic (Paladur, Kulzer and Co., GmbH) to the bone surrounding the craniotomy. For the duration of the experiment the cortex was covered with agar and a superficial layer of wax. The animal's head was adjusted so that it had an unobstructed view of a target screen placed 1 metre in front of its eyes. The pupils were dilated with drops of 1% atropine sulphate (Atropin, Sigma Co., Ltd.) and the meningeal coverings were retracted with 10% phenylephrine hydrochloride (Neosynephrin, Winthrop Laboratories). Plastic contact lenses of zero power were fitted to prevent corneal drying. Binocularly was used to determine the value of appropriate correcting lenses.

MATERIALS AND METHODS

Animal Preparation

Experiments were performed on 15 adult cats, each weighing between 2 and 5 kg. Each cat was anaesthetised for surgery with intramuscular injections of ketamine hydrochloride ("Ketalar", Parke Davis, 20 mg/kg) plus 0.3 cc of the muscle relaxant, xylazine ("Rompun", Bayer Aust. Ltd.). The saphenous vein was cannulated, a tracheotomy performed and a cannula inserted. Eye movements were reduced to a minimum by complete paralysis of the animal combined with bilateral cervical sympathectomy (Rodieck et al, 1967; Kinston et al., 1969). The animal was then placed in a stereotaxic instrument, and a stainless steel screw which served as an EEG electrode was embedded in the skull above the frontal lobe. A craniotomy was made at Horsley-Clarke (HC) co-ordinates 0 to -5.0mm anterior-posterior, and 10.0mm to 15.0mm medial-lateral, the dura reflected, and a circular well was cemented with dental acrylic ("Paladur", Kulzer and Co.GmbH) to the bone surrounding the craniotomy. For the duration of the experiment the cortex was covered with agar and a superficial layer of wax. The animal's head was adjusted so that it had an unobstructed view of a tangent screen placed 1 metre in front of its eyes. The pupils were dilated with drops of 1% atropine sulphate ("Atropt", Sigma Co. Ltd.), and the nictitating membranes were retracted with 10% phenylephrine hydrochloride ("Neo-Synephrine", Winthrop Laboratories). Plastic contact lenses of zero power were fitted to prevent corneal drying. Retinoscopy was used to determine the value of appropriate correcting lenses.

Animals were paralysed with an initial intravenous injection of gallamine triethiodide ("Flaxedil", May and Baker Aust. Pty. Ltd) followed by a continuous intravenous infusion of "Flaxedil" (infusion rate of 5 mg/kg/hr), pancuronium bromide ("Pavulon" N.V. Organon Oss Holland; infusion rate 0.4 mg/kg/hr) and sodium pentobarbitol ("Nembutal" Bomac Laboratories Pty. Ltd; infusion rate 1.0 mg/kg/hr; see Hammond, 1978) in compound sodium lactate ("Hartmann's injection Abbott Australasia Pty. Ltd.). Throughout the experiment the animal was ventilated artificially with a mixture of 70% nitrous oxide and 30% oxygen. Heart rate and brain activity were monitored throughout the experiment as a guide in maintaining an appropriate level of anaesthesia. The end-tidal CO₂ level was monitored continuously with a Datex Normocap medical gas analyser and maintained at between 3.0 and 4.0%. Normal body temperature of 38 degrees was maintained using a heated blanket.

Visual Stimulation and Recording

The optic disks and areas centralis were projected and plotted on the tangent screen. Extracellular single unit recordings were made with Merrill-Ainsworth (Merril and Ainsworth, 1972) or Levick (Levick, 1972) type microelectrodes with an impedance of 2-10 M Ω . For each visually responsive cell the activating region of the receptive field was plotted in the form of minimum response fields (Bishop and Henry, 1972) by presenting a moving or flashing stimulus well outside the receptive field and gradually approaching its centre. The borders between responsive and unresponsive regions were then drawn directly on the tangent screen. Receptive field position in the visual field was referenced to the projection of the area centralis (Bishop et al., 1971).

A visual display system and data collecting routines controlled by a Hewlett-Packard MX computer (Bullier et al., 1982) were used to make quantitative

assessments of a number of response properties. Most of the experimental procedures used in this evaluation have been set-out in earlier publications from this laboratory cited below. Measurements were made of the dimensions of the receptive field mapped with the aid of either stationary flashing or moving stimuli. Responses were interpreted as excitatory or inhibitory when the firing rate increased or decreased, respectively (Henry et al., 1969; Bishop et al., 1971a; Bishop and Henry, 1972; Henry, 1977). The following battery of tests was used to assess the cells' response properties:

(a) Orientation specificity was measured in the form of a tuning curve prepared from the responses obtained by inter-leaving light or dark bar stimuli of different length at different test orientations (Henry et al., 1974a).

(b) Directional selectivity was assessed from the discharge frequency recorded in the peaks of average response histograms which resulted when a bar of light or a dark edge moved forward and backward across the receptive field in the axis of the cell's preferred orientation (Henry et al., 1974b).

(c) A direct assessment of flanking inhibition in the receptive field was made by investigating a cell's average response to a stimulus sweeping the receptive field whilst a conditioning stimulus maintained a background of induced activity.

(d) Velocity tuning and highest effective velocity was determined by gradually increasing and then decreasing the velocity of an optimal stimulus. Cut-off velocity was defined as the highest speed capable of modulating the neuronal discharge (Goodwin and Henry, 1978).

(e) Length summation was calculated from the region of the receptive field in which lengthening the stimulus continued to increase the strength of the excitatory response (Henry et al., 1978).

(f) Summation of excitation to test for the existence of linearity of response summation was assessed by adding the excitatory responses from two parts of the receptive field (Henry, et al., 1978).

(g) Binocular interaction fields were prepared from the responses obtained by spatially overlapping the receptive fields of each eye (Pettigrew et al., 1968; Bishop et al., 1971)

Responses were initially judged by listening to an audio monitor and viewing an oscilloscope. For many cells poststimulus time histograms were made on-line to verify the qualitative impressions and to document in quantitative form particular examples of receptive field properties.

Reconstruction of electrode tracks

In the course of each electrode penetration small electrolytic lesions (5 microamps for 5 sec.) were made at regular intervals.

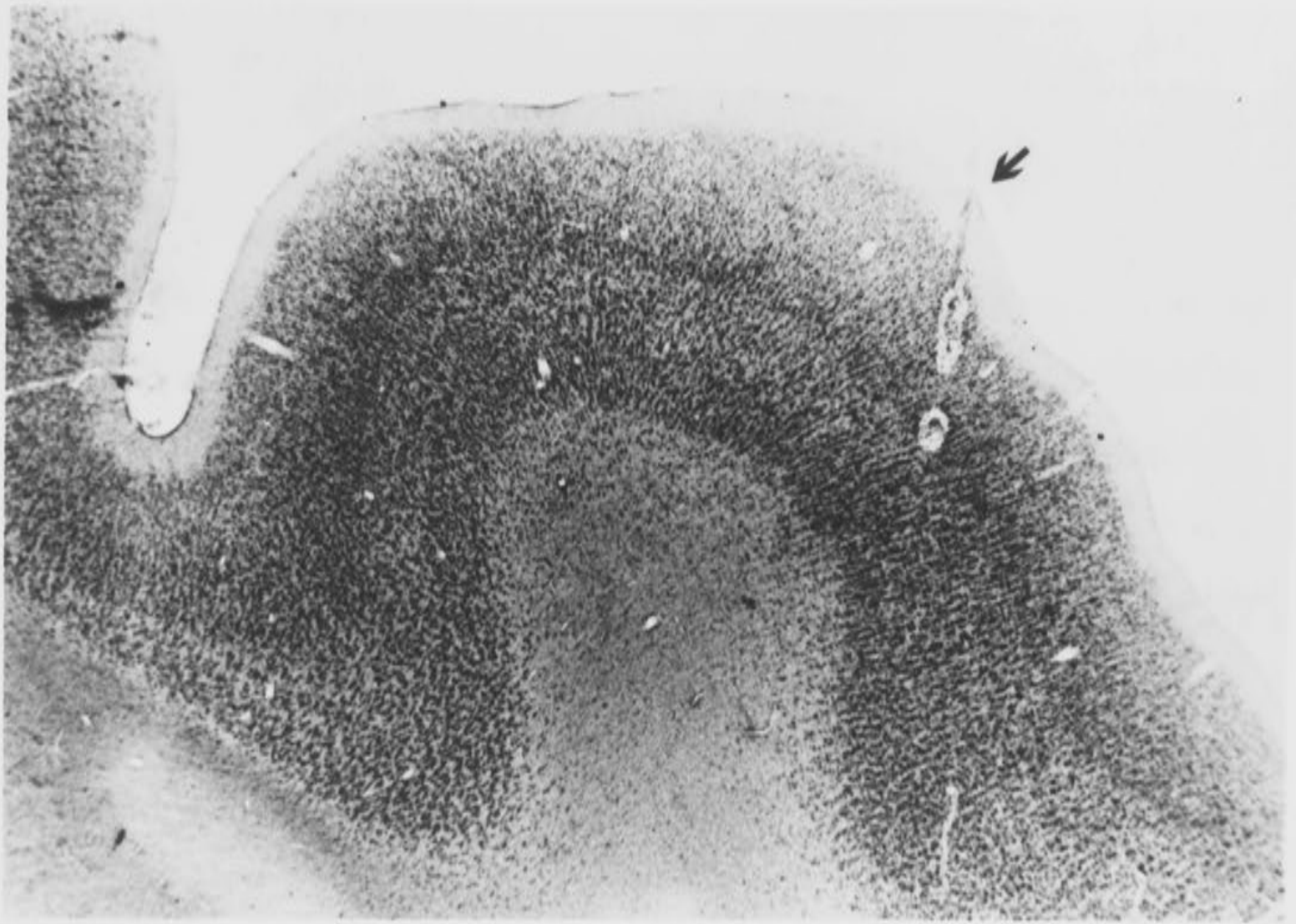
Perfusion

At the end of the experiment the animals were deeply anesthetised with an intravenous injection of 2 cc of sodium pentobarbitol ("Nembutal") and were

perfused transcardially with solutions of normal 0.9% saline and 10% neutral buffered formol saline (NBFS). The brain was then removed, left for 24 hours in 10% NBFS, blocked and sectioned at 50 μ m on a freezing microtome. Selected sections were counterstained with cresyl violet or neutral red and inspected for evidence of lesion sites and the subsequent reconstruction of the electrode track. An example of a typical electrode track is illustrated in Fig. 4.1. This illustrates a coronal section of area 21a showing an electrode track with three electrolytic lesions.

FIGURE 4.1

Photomicrograph of coronal section of area 21a showing electrode track (indicated by arrow) with three electrolytic lesions. The section has been stained with neutral red in order to reveal the pattern of cortical lamination.



1mm

RESULTS

The sample

Results were obtained from a sample of 115 cells isolated in area 21a. At the outset, the location of area 21a was determined from its relationship to the suprasylvian sulcus and the lateral sulcus. As the investigation progressed, however, the location could be confirmed from the central retinotopic distribution of the recorded receptive fields (Tusa and Palmer, 1980) and also from the distinctive response pattern that characterises most, if not all, of the cells in area 21a (see below). These two criteria were used only to confirm the location of the area and care was taken to make sure their application did not restrict the detection of cell variety in area 21a.

Of the 115 cells sampled, 58 were subject to detailed quantitative analysis and although the remainder were assessed from their responses to hand-held stimuli it was usually possible to make effective estimates of the receptive field dimensions, the preferred orientations and the total width of the orientation tuning curves assessed from the point of the null response.

Retinotopic projection

Figure 4.2a provides a display of the visual field location of the receptive fields of all 115 cells represented by the midpoints of their receptive fields. Figure 4.2b

FIGURE 4.2

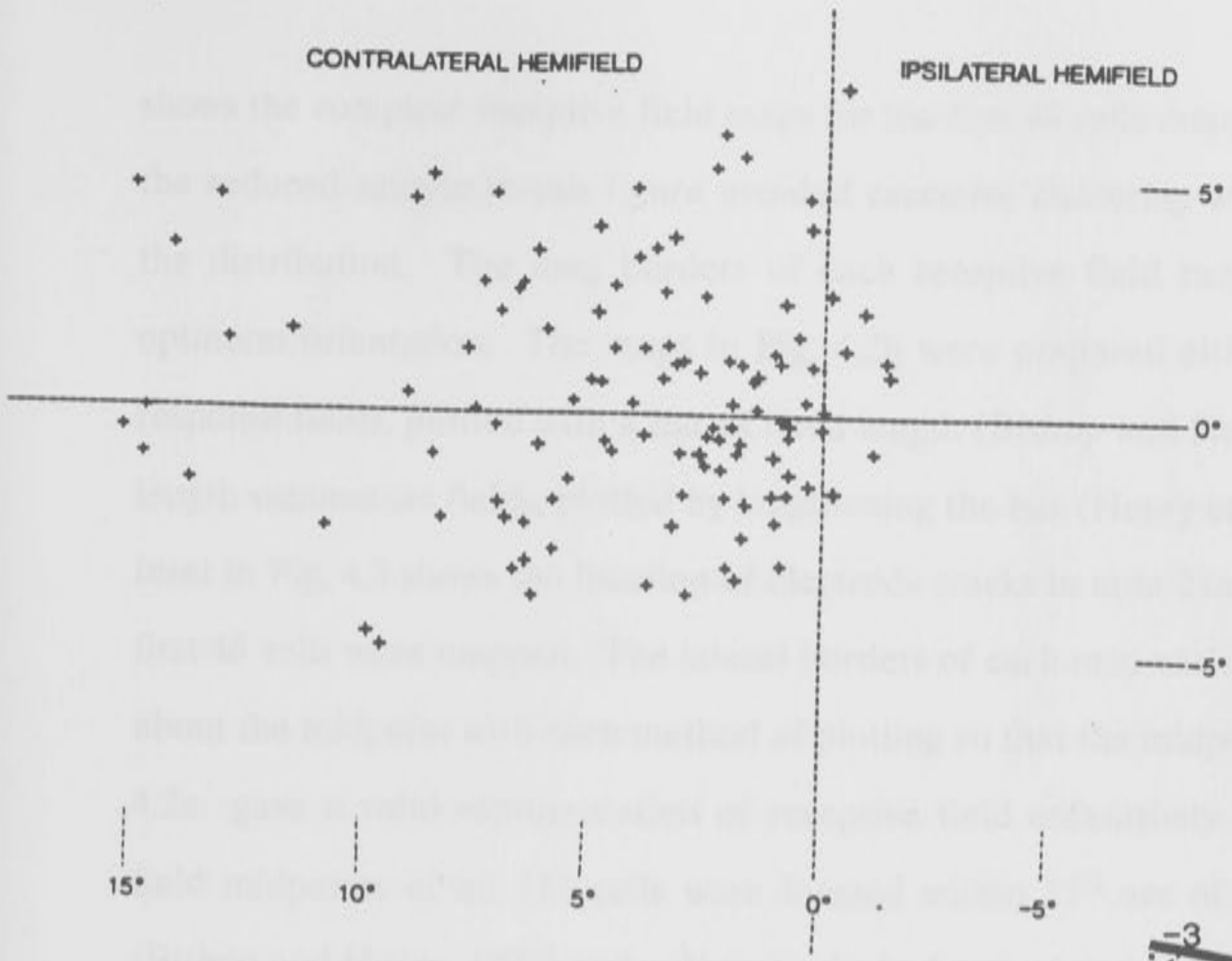
Spatial distribution of receptive fields of area 21a cells.

(A) Visual field projection of 115 cells represented by the mid-points of their discharge regions.

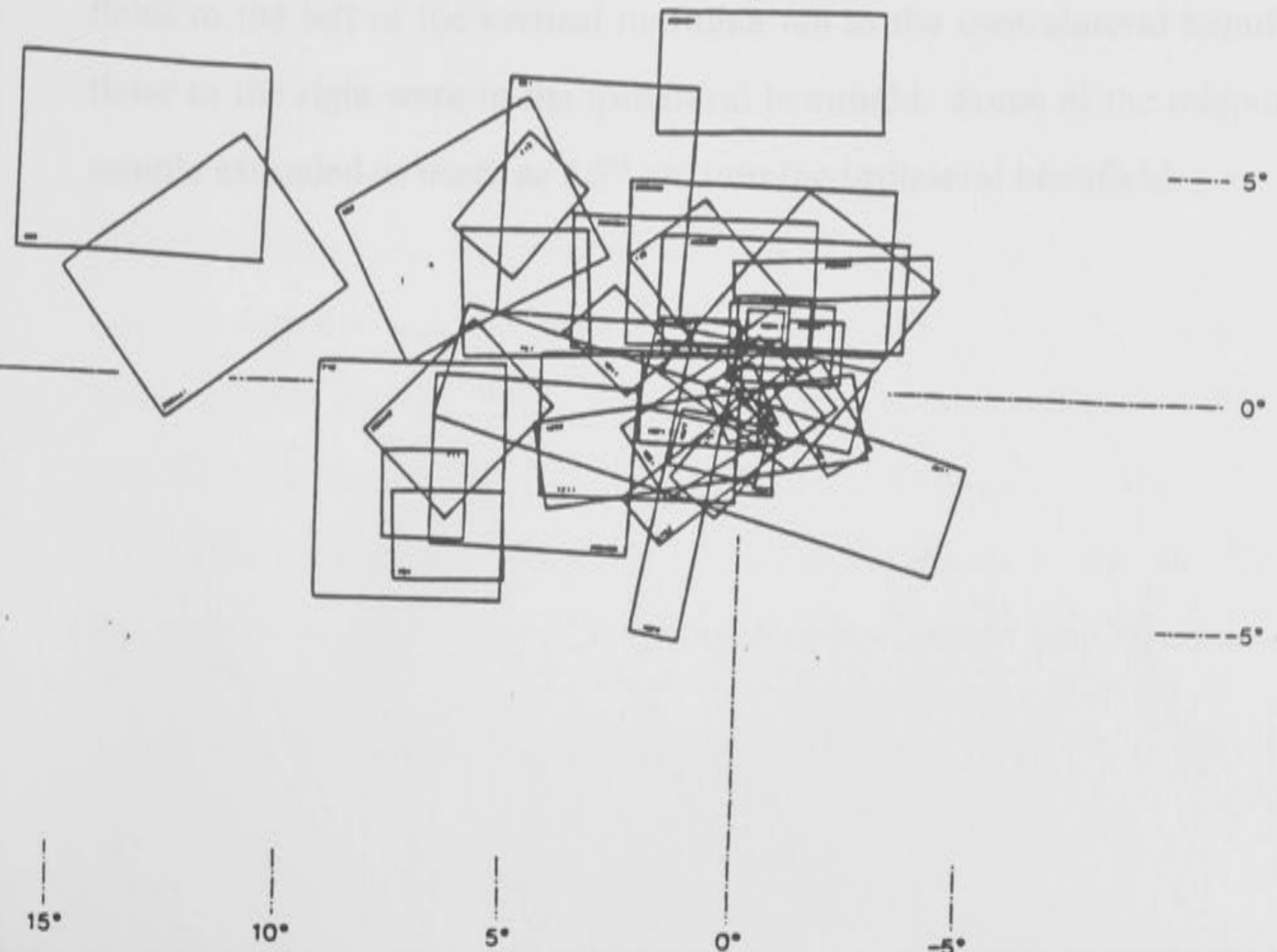
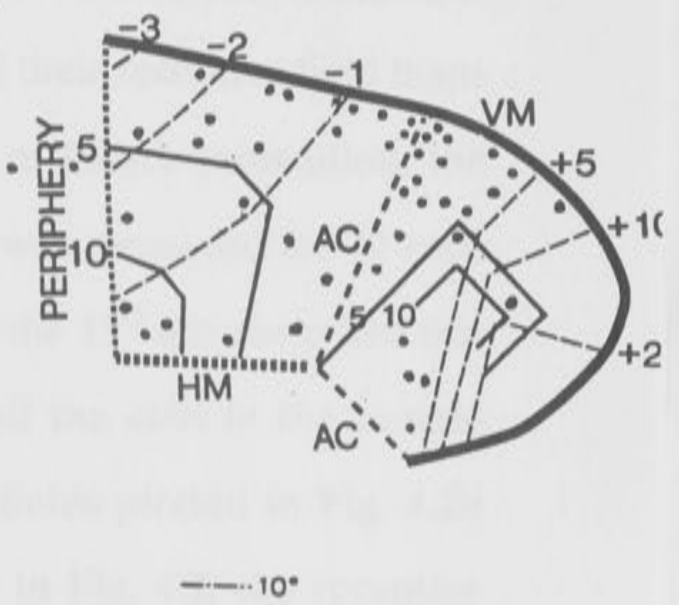
(B) Maps of discharge regions of 48 cells plotted either as minimum response fields or length summation fields. Long borders run parallel to optimum orientations. The majority of receptive fields are located within 10 degrees of the visual axis and a small proportion extends into the ipsilateral hemifield.

Inset is a schematic illustration of area 21a based on the topographical map of Tusa and Palmer (1980) showing the location of electrode penetrations. Dotted lines = elevations; solid lines = azimuths; AC = area centralis; VM = vertical meridian; HM = horizontal meridian.

(A)



(B)



shows the complete receptive field maps for the first 48 cells mapped in the study; the reduced sample in this figure avoided excessive cluttering without distorting the distribution. The long borders of each receptive field run parallel to the optimum orientation. The maps in Fig. 4.2b were prepared either as minimum response fields, plotted with a bar of fixed length (Bishop and Henry, 1972) or as length summation fields, plotted by lengthening the bar (Henry et al., 1978). The inset in Fig. 4.2 shows the location of electrode tracks in area 21a from which the first 48 cells were mapped. The lateral borders of each map varied symmetrically about the midpoint with each method of plotting so that the midpoint plots in Fig. 4.2a gave a valid representation of receptive field eccentricity. The receptive field midpoints of all 115 cells were located within 15° arc of the visual axes (Bishop and Henry, 1972) and only two cells had part of their receptive field maps extending beyond the 15° arc circle. The full extent of length summation, the method that produces the widest receptive field maps, was measured for 12 cells only. More receptive fields may have extended beyond the 15° arc circle had this method of mapping been applied more frequently. All the cells in the sample were binocularly driven and the monocular receptive fields plotted in Fig. 4.2b were all for the dominant eye. In both of the graphs in Fig. 4.2, the receptive fields to the left of the vertical meridian fell in the contralateral hemifield while those to the right were in the ipsilateral hemifield. Some of the midpoints in the sample extended as much as 1.5° arc into the ipsilateral hemifield.

Receptive field topography:

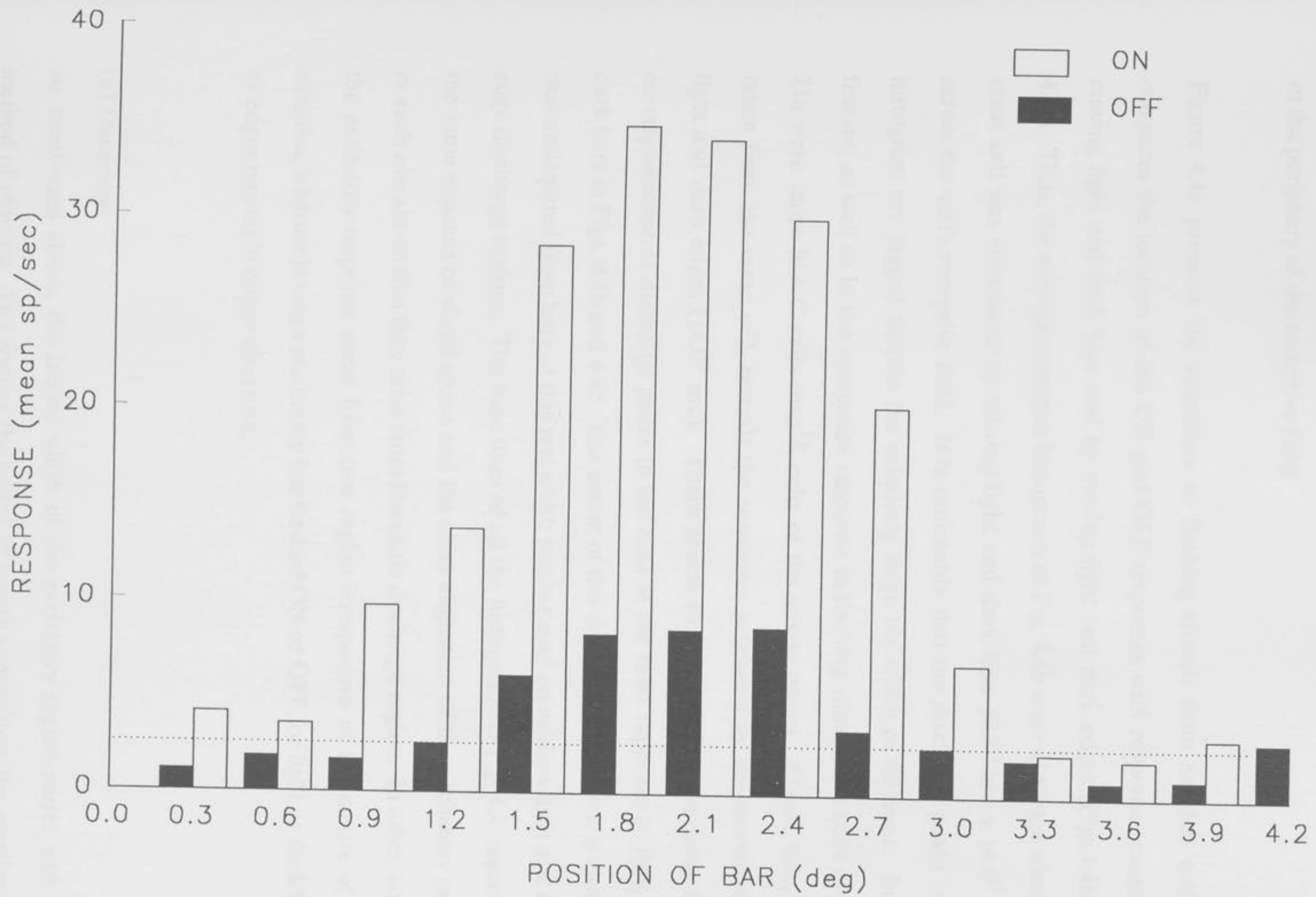
(1) Excitatory regions

(i) *Disposition*

The receptive fields plotted in Fig. 4.2b all recorded the extent of excitatory regions and do not include any representation of inhibitory regions. The excitatory regions, or the areas where the stimulus caused an increment in the firing rate, were pre-eminent in the receptive fields of all cells in the sample. There were few cells that showed any evidence of inhibition, or a decrement in the firing rate in the presence of the stimulus. The excitatory regions of the receptive fields were generally plotted with moving bars or edges and for most cells the dimensions of this plot corresponded closely to that obtained with stationary flashing stimuli. The response to a flashing bar consisted of a composite ON/OFF discharge from all points in the excitatory region and generally, as mentioned earlier, this constituted the entire receptive field. Most cells responded weakly to small flashing spots of light although a stationary flashing bar of light at the cell's preferred orientation was usually an effective stimulus. Figure 4.3 is an average response histogram which shows a representative example of the ON (white bars) and OFF (dark bars) response profiles (mean level of spontaneous activity shown by horizontal broken line) gathered from a stationary flashing bar (0.6° arc \times 14.0° arc) in which each test point is one bar width removed from its neighbour. As is the case for C or complex cells with a uniform receptive field in the striate cortex (Hubel and Wiesel, 1962; Henry, 1977), the ON and OFF responses were spatially coincident and displayed a profile that peaked towards the centre of the excitatory region. For most cells the strength of the same response, either the ON or the OFF, was

FIGURE 4.3

Average response histograms showing ON and OFF responses to stationary flashing bars. The dotted line represents the level of spontaneous activity. Response axis is common to both histograms.



greater at all test points. Occasionally, either the ON or OFF response faded out in the periphery of the receptive field.

Figure 4.4a presents the responses to flashing stimuli from another cell and compares the location of the ON and OFF responses with responses caused by moving light and dark bars and by moving light and dark edges (Figs. 4.4b and 4.4c). Thus, the average response histograms in Fig. 4.4b were obtained when the same cell was stimulated by moving light and dark bars (0.3° arc x 14.0° arc) across the cell's receptive field. It is noticeable that the discharge peaks in the histogram are jagged despite the relatively large bin width of 80 msec. In this feature, as well as in the composite response to flashing stimuli, the cells of area 21a were more like C cells than S cells of the striate cortex. Figure 4.4c, also taken from the same cell, records the responses produced by the movement of light and dark edges (14.0° arc). There seems to be a small move away from correspondence of discharge peaks in the case of the cell's responses to light and dark bars in Figs. 4.4b and 4.4c. The extent of this difference, however, is close to that anticipated from bars of this test width passing over coincident light and dark edge discharge regions. The base lines of all the histograms in Fig. 4.4 represent the same expanse of visual space and the close alignment of the excitatory peaks in each row shows that they arise from the same discharge region. In other words, the excitatory response came from one region irrespective of the nature of the stimulus, whether it was a stationary bar flashed ON or OFF, or light or dark bars or edges moved in either direction.

(ii) *Dimension*

As mentioned above, the lateral width of the excitatory region varies with the method of plotting. The greater the extent of length summation, the smaller will

FIGURE 4.4

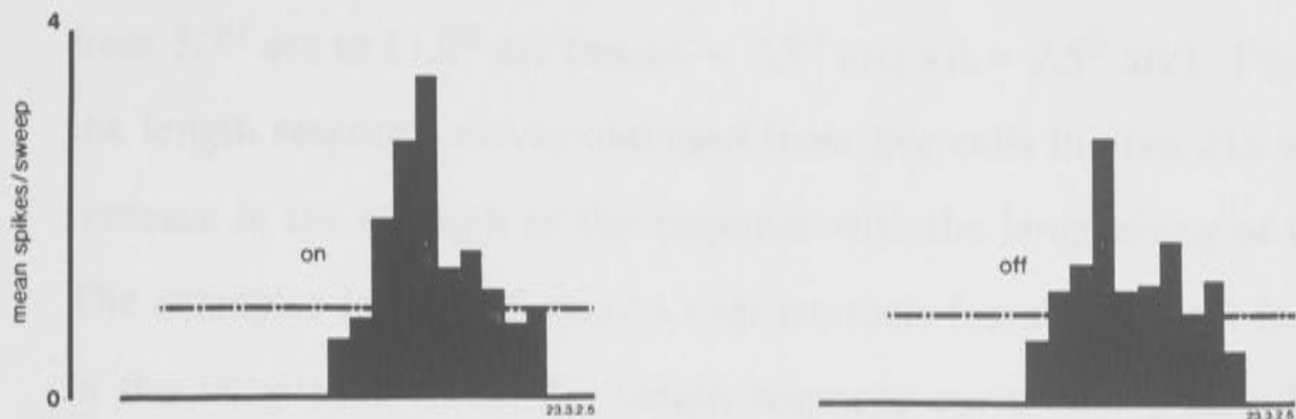
Average response histograms to different types of visual stimulation.

(A) Response to a stationary bar flashed ON (1 sec.) and OFF (1 sec.) for 17 presentations. Dotted line indicates level of spontaneous activity.

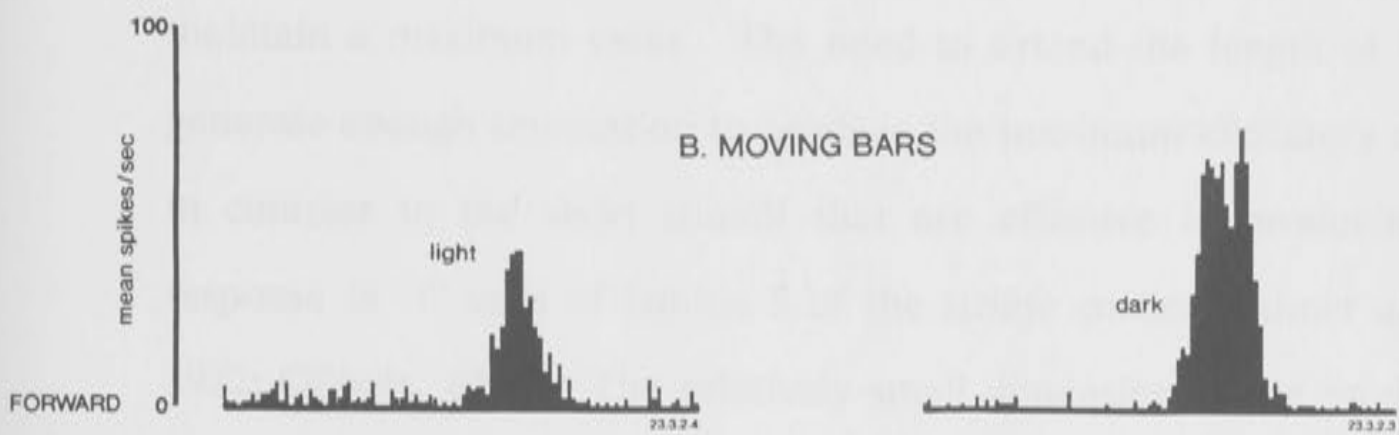
(B) Average response histograms to light and dark bars moved in forward and reverse directions.

(C) Average response histograms to light and dark edges moved in forward and reverse directions. Base lines represent the same part of visual space and there is spatial correspondence of discharge peaks in each histogram. The time scale applies only to histograms for moving stimuli.

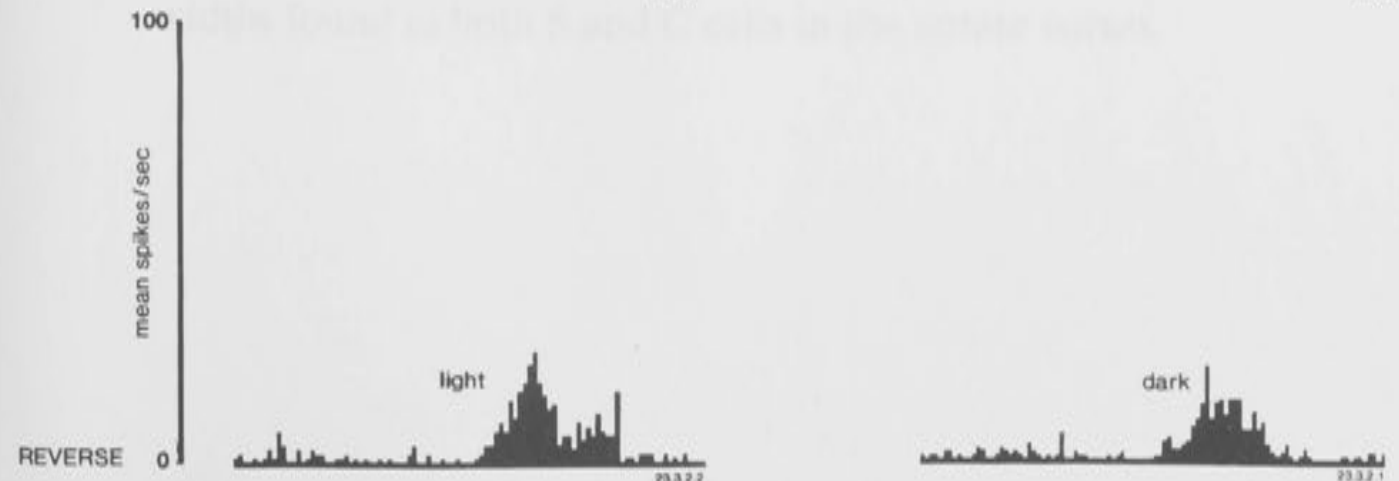
A. FLASHING BAR



B. MOVING BARS



C. MOVING EDGES



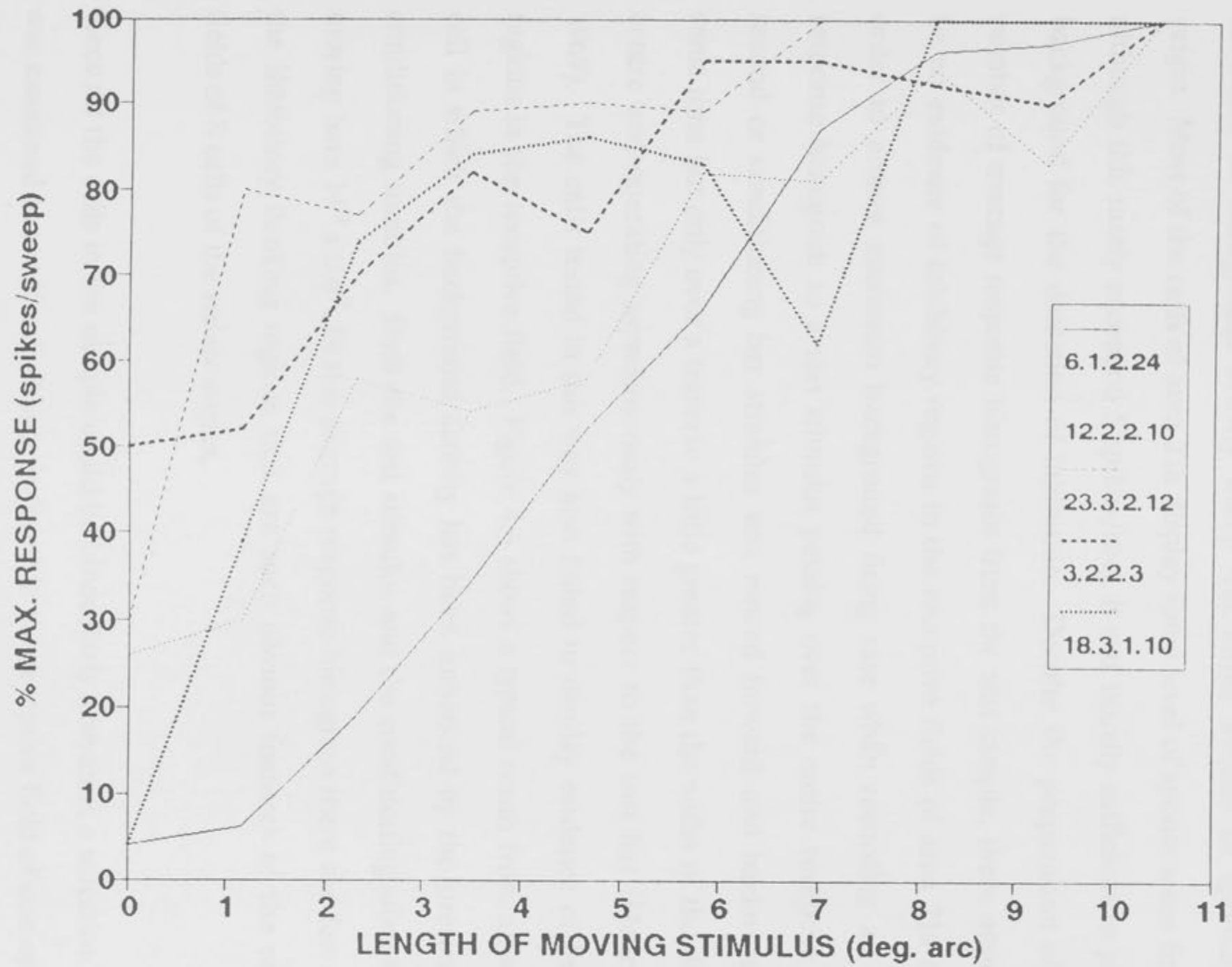
2 sec
3 deg.arc

be the minimum response field (Bishop and Henry, 1972). For the 12 cells evaluated quantitatively by extending the ends of the optimally-oriented moving bar to the point where the response ceased to increment, the lateral width ranged from 3.7° arc to 11.6° arc (mean = 7.5° arc; s.d. = 2.5° arc). Figure 4.5 contains the length response curves obtained from five cells in area 21a which shows the increase in the strength of the response with the lengthening of the moving bar. The examples in Fig. 4.5 show a characteristic feature, present in almost all cells in the sample, in which the length response curve rose gradually to reach and maintain a maximum value. The need to extend the length of the stimulus to generate enough summation to produce the maximum excitatory response stands in contrast to the short stimuli that are effective in producing a maximum response in C cells of lamina 5 of the striate cortex (Palmer and Rosenquist, 1972; Gilbert, 1977). The relatively small dimension of the minimum response field for area 21a cells reflects this summation requirement and the mean value of this plot in a sample of 37 cell (hand plotted) was 3.0° arc (s.d. = 1.5° arc).

The separation of the primary borders (fore to aft) was determined either from the width of the response peak in the average response histogram or by listening to the response to hand-held stimuli. Where the first method was used, the primary width was assessed as comprising that part of the average response histogram which exceeded the level of the cells spontaneous firing. For a sample of 108 cells the primary width ranged from 0.7° arc to 4.9° arc (mean = 2.1° arc; s.d. = 0.9° arc). It should be noted that this range extends into the range of widths found in both S and C cells in the striate cortex.

FIGURE 4.5

Length response curves prepared for 5 area 21a cells showing the increase in the strength of the response with the lengthening of a moving bar. The length of the bar producing the optimum response is generally in excess of 2 deg.arc. and there is little evidence of inhibition with longer bars.



STIMULUS: LIGHT BAR 0.3 deg.arc wide

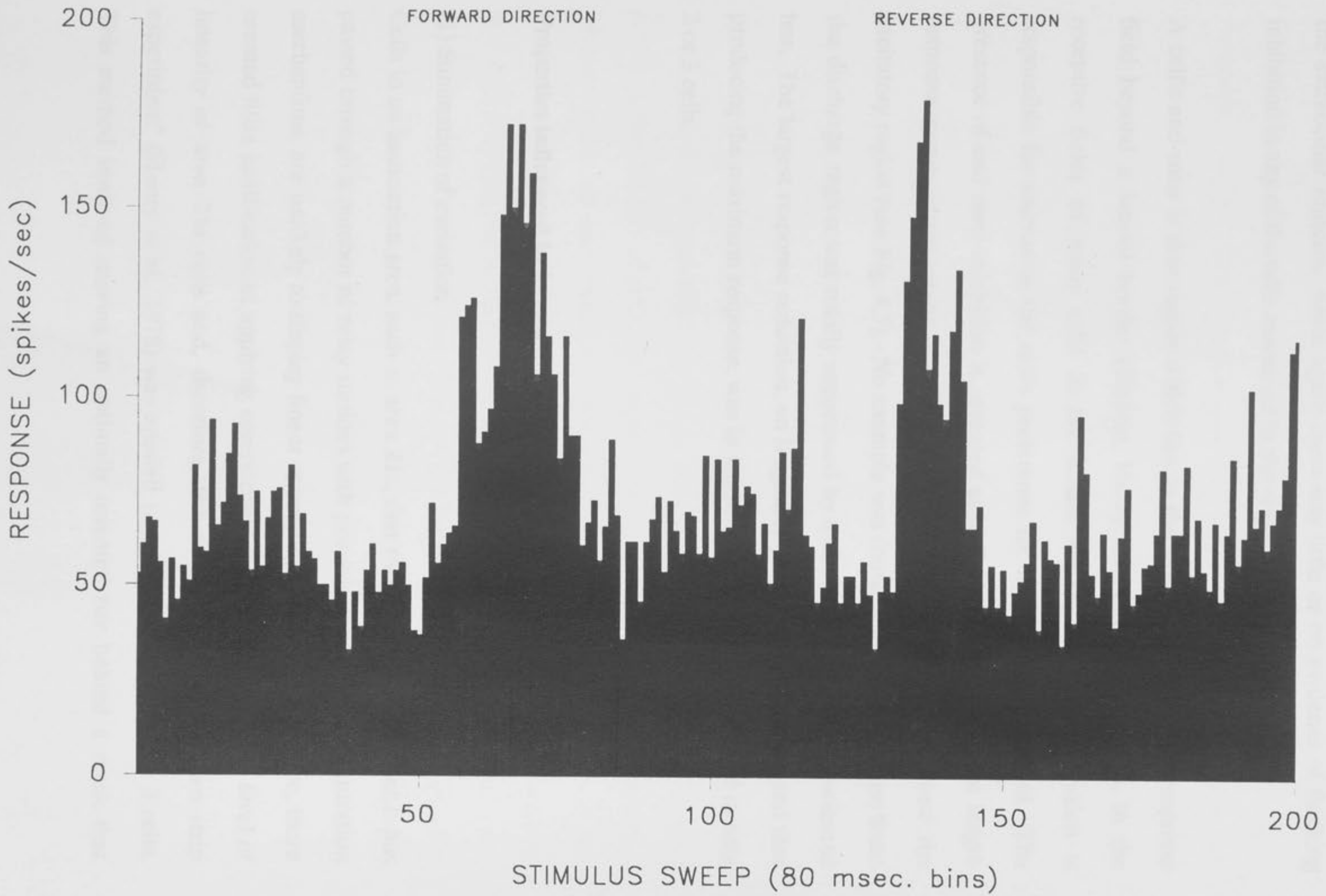
(2) Inhibitory regions

Since extracellular recording was used, inhibition was recognised through the reduction of background activity which was either evoked or spontaneous in origin. Most of the cells of area 21a display some level of spontaneous firing and, although this rarely exceeded 5 spikes/sec., it was usually sufficient to provide a background for the detection of inhibition. Despite the preparation of a large number of average response histograms from the test sample, there appeared to be no evidence of inhibitory regions in the receptive fields of area 21a cells. In order to ensure maximum background firing rate when recording an average response histogram to a test stimulus passing over the entire receptive field, a second or conditioning bar stimulus was moved forward and backward at the same time but only over a traverse a little greater than the width of the discharge centre and operating asynchronously with respect to the test bar (Henry et al., 1969). The cells tested in this way also failed to display evidence of inhibitory regions in the receptive field. Figure 4.6 shows a typical result from an area 21a cell in which the background activity has been enhanced by the presence of a conditioning stimulus. Both the test stimulus and the conditioning stimulus were moving bars $14^{\circ} \times 0.6^{\circ}$. In this average response histogram there are few signs of the inhibitory flanking regions that are such obvious features of the receptive fields of S cells of the striate cortex.

Since all the cells in the sample could be binocularly activated, a selection of cells was examined to see if a discharge induced in the receptive field of one eye could be suppressed through an inhibitory region in the receptive field of the other eye i.e. binocular conditioning (Henry et al., 1969). This was achieved by passing the test bar over the receptive field of one eye whilst simultaneously moving a conditioning stimulus over the receptive field of the other eye. The independent

FIGURE 4.6

Average response histogram for area 21a cell in which the background activity has been enhanced by the presence of a conditioning visual stimulus. The test stimulus (a moving bar: 14.0 x 0.6 deg.arc) produced a non-direction selective discharge but typically showed only the slightest sign of inhibitory flanking regions.



stimulation of the two eyes was ensured by placing a septum at right angles between the eyes, or by increasing with prisms, the divergence due to paralysis of the extraocular muscles. Once again there was little or no evidence of flanking inhibition in any of the cells examined in this way.

A cell's end-zone is that region which lies to the side of the minimum response field beyond a lateral border (Bishop, Henry and Smith, 1971) and, in the receptive fields of many cells in the striate cortex, end-zone inhibition is responsible for increasing the cell's preference for fore-shortened stimuli. The presence of end zone inhibition is revealed as a dip in the profile of the length response curve at the point where the bar stimulus is extended beyond the excitatory region (see Fig. 4.5). No example was found where the response from the discharge region was totally suppressed by extending the optimally-oriented bar. The largest response reduction, on lengthening the bar stimulus beyond that producing the maximum response, was in the order of 25%, and occurred in only 2 or 3 cells.

Properties influenced by interactions within the receptive field:

(1) Summation of excitation

Cells in an association area, such as area 21a, that receive their input after it has passed through a number of relay stations with possible threshold and saturation mechanisms, are unlikely to display linear summation. As a consequence, there seemed little justification in applying complicated methods to assess the level of linearity of area 21a cells and, therefore, the conceptually simple "two strip experiment" (Henry et al., 1978) was applied to a small sample of only 3 cells. This method involved moving an optimally oriented bar behind a mask that

exposed two narrow strips that ran across the receptive field at right angles to the direction of preferred orientation. By appropriate lengthwise positioning of the bar it was possible to expose the moving bar through one strip (response = R_1), and then through the second strip (response = R_2) and finally through both strips (response = $R_{1,2}$). This experiment could only be applied to those cells that responded to short bars, but in each of the 3 cells tested the result was the same, being similar to that shown in Fig. 4.7. In this experiment the two exposed strips of the receptive field were 0.57° wide and separated by 0.57° . At each of the three stimulus contrasts (contrast = $(L_{\max} - L_{\min}) / (L_{\max} + L_{\min})$, where L is the luminance), the sum of the two individual responses ($R_1 + R_2$) was always greater than the combined response ($R_{1,2}$), indicating the absence of linear summation. This consistent result could come from saturation in the combined response but this seemed unlikely with the use of short bars of low contrast.

(2) Orientation specificity

In the past, experiments involving the measurement of acuteness of orientation tuning curves for bars of different length have been used to study the interaction between excitatory and inhibitory regions in the receptive fields of cells in the striate cortex. It has been argued that the inhibition arising from regions flanking the excitatory region is responsible for sharpening the tuning for stimulus orientation (Henry et al., 1974a). The shorter bars that fail to protrude into the inhibitory flanks produce broader orientation tuning curves than their longer counterparts. For comparative purposes, therefore, the test bar should be long enough to encompass the entire receptive field. Orientation tuning curves were prepared for 48 cells of area 21a using a long light bar (0.3° arc x 14.0° arc) moving at optimal velocity. The half width at half height values for these curves ranged from 8° arc to 29° arc (mean = 15.6° arc; s.d. = 5.3° arc). A distribution histogram of these data is presented in Fig. 4.8 which shows the variation in

FIGURE 4.7

Examples of the 'two strip' experiment applied to two area 21a cells in order to test for spatial summation of response in two strips of receptive field (0.57 deg.arc wide and separated by 0.57 deg.arc) running perpendicular to the optimal stimulus orientation. A mask formed the strips in front of a dark bar which moved first along one strip (response: R_1) then the other (R_2) and then both together ($R_{1,2}$). The requirement for linearity ($R_{1,2} = R_1 + R_2$) was not met at any test contrast level. All responses exclude spontaneous activity. The lowest background luminance was 17.0 cd/m².

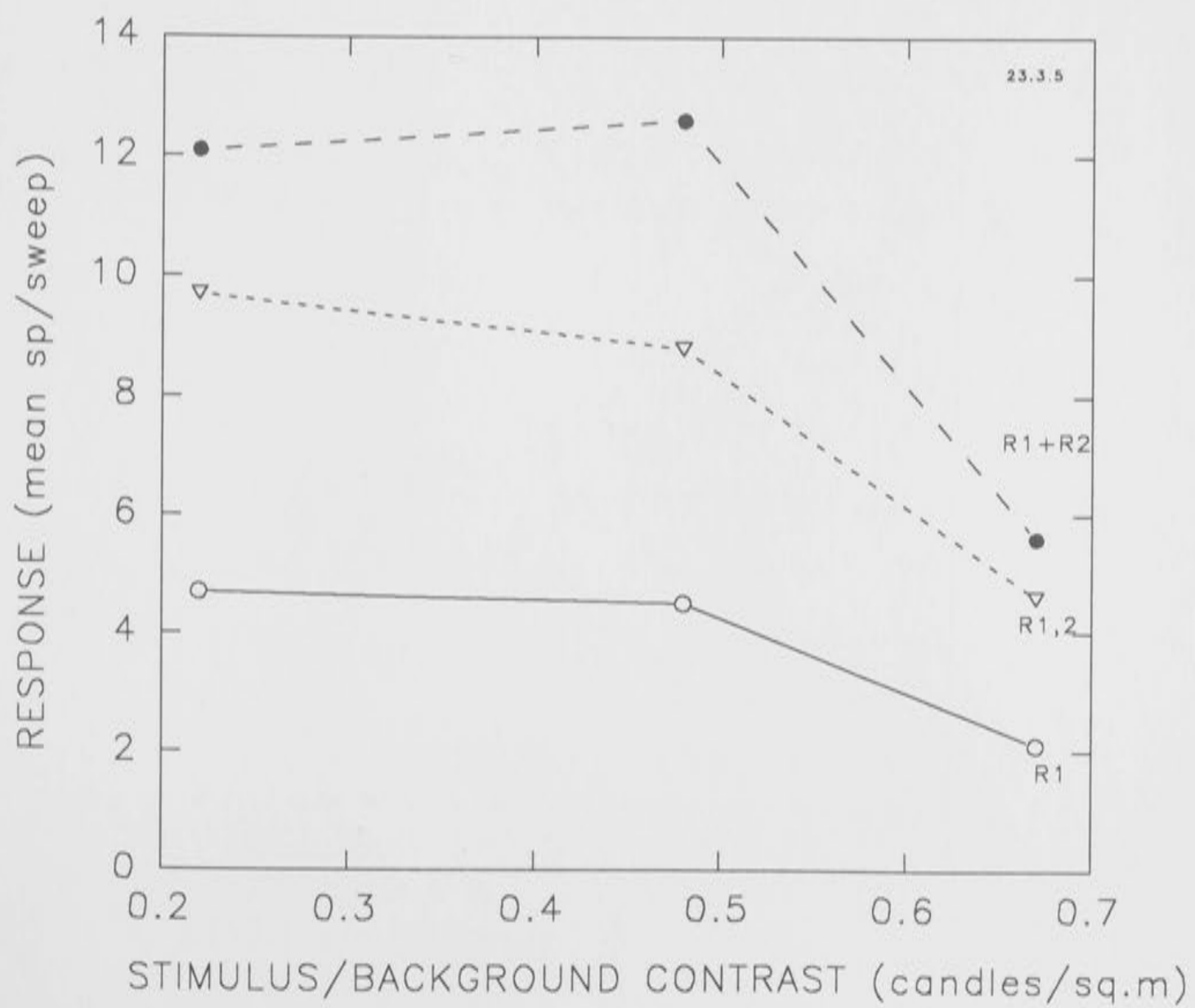
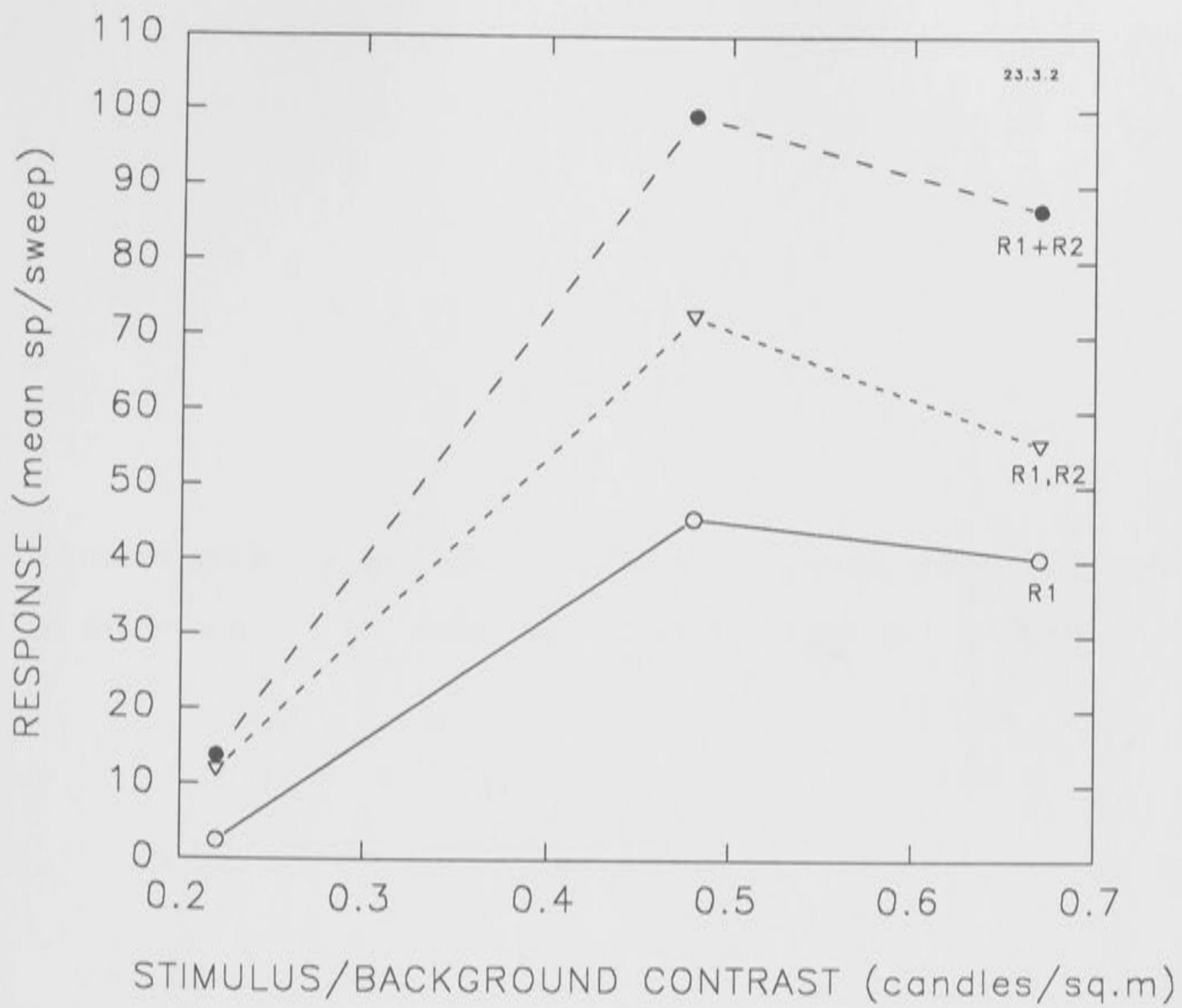
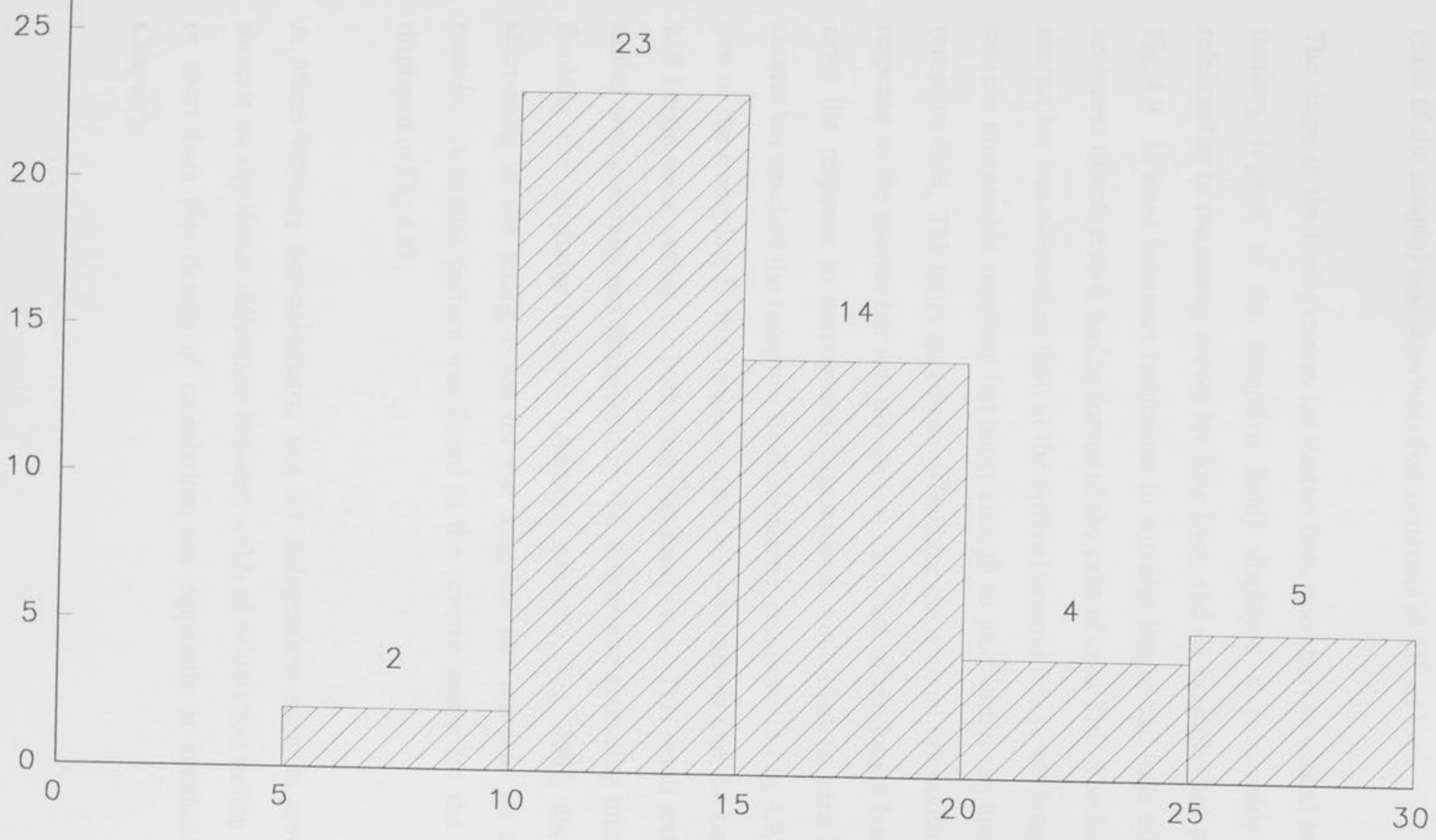


FIGURE 4.8

Distribution histogram showing the variation in orientation specificity, measured as the $1/2$ width at $1/2$ height of the tuning curve, in a group of 48 cells.

NUMBER OF CELLS



ACUTENESS OF TUNING TO ORIENTATION
HALF WIDTH AT HALF HEIGHT (deg)

orientation specificity for the 48 cells. From these data it can be seen that 25 cells (52% of the sample) had an orientation acuteness of 15° or less.

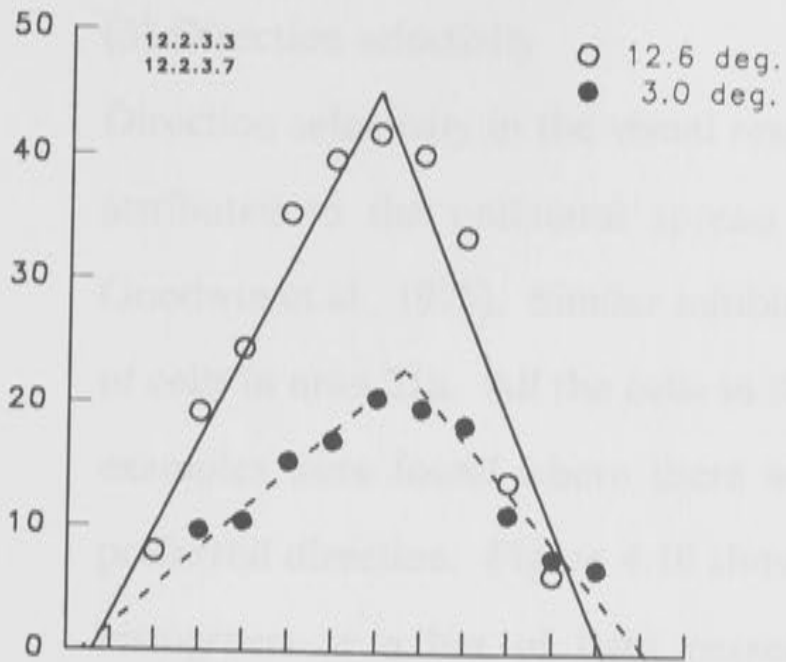
The shape of the tuning curves for shorter bars (too short to extend into possible flanking regions of the receptive field) displayed a reasonably consistent relationship to the tuning curves for long bars, and six examples are produced in Fig. 4.9. In most instances reductions in stimulus length had little effect on the acuteness of orientation tuning curves of the cells of area 21a. The length of the shorter bar was selected so that, at the optimal orientation, it was long enough to cause a measurable response but short enough to pass within the bounds of the receptive field. The latter qualification held for the optimal orientation since the response to the shorter bar was always less than that of the longer bar and in all cases the response to non-optimal orientations diminished to zero before the shorter bar reached the receptive field boundary. As shown in Fig. 4.9, for all but one of the examples, E, the range of effective orientations and the half width at half height were similar for both long and short bars. This result indicates that tuning was generally not sharpened by the longer bar extending into the areas flanking the discharge region. Another example, Fig. 4.9E, does show a sharpening of the tuning curve for the long bar but this was an uncommon pattern. A similar pattern was found in the reverse sweep for the same cell, displayed in Fig. 4.9F.

A Mann-Whitney non-parametric test for independent samples revealed that there is no significant difference between width of orientation tuning using long or short bars (for details of calculation, see Appendix at conclusion of this Chapter).

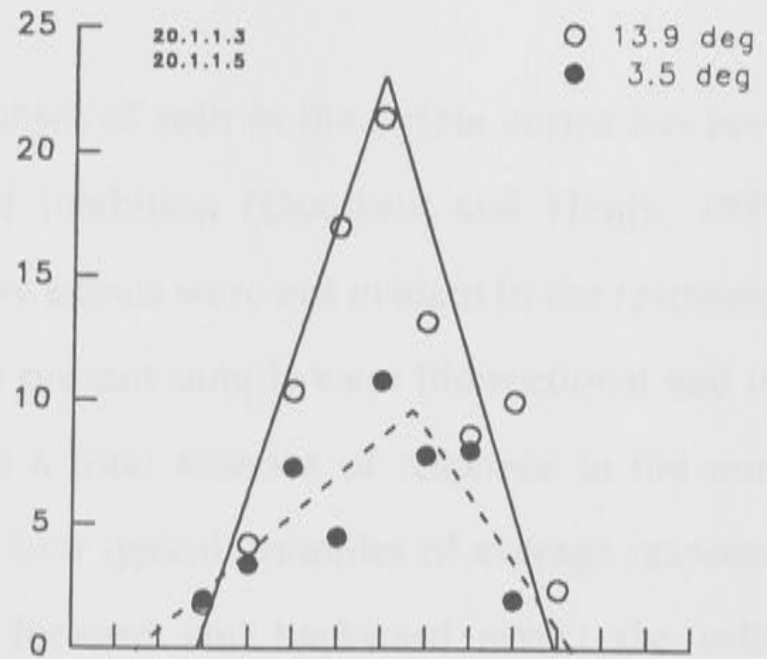
FIGURE 4.9

Regression curves fitted to orientation tuning plots, for long and short light bars, obtained from 5 cells in area 21a. Bar lengths in each diagram are given in degrees of arc. For the 4 cells represented in Figs. 4(A) to 4(D), the 1/2 width at 1/2 height, in each example, is similar for the two curves and the tuning does not become broader with the short bar as is the case for S cells in the striate cortex (Henry et al., 1974a). Figs. 4(E) and (F) display results, for one cell, of forward and backward sweeps of the stimuli. Note that this is one of the few examples where the longer bar resulted in a sharpening in orientation tuning.

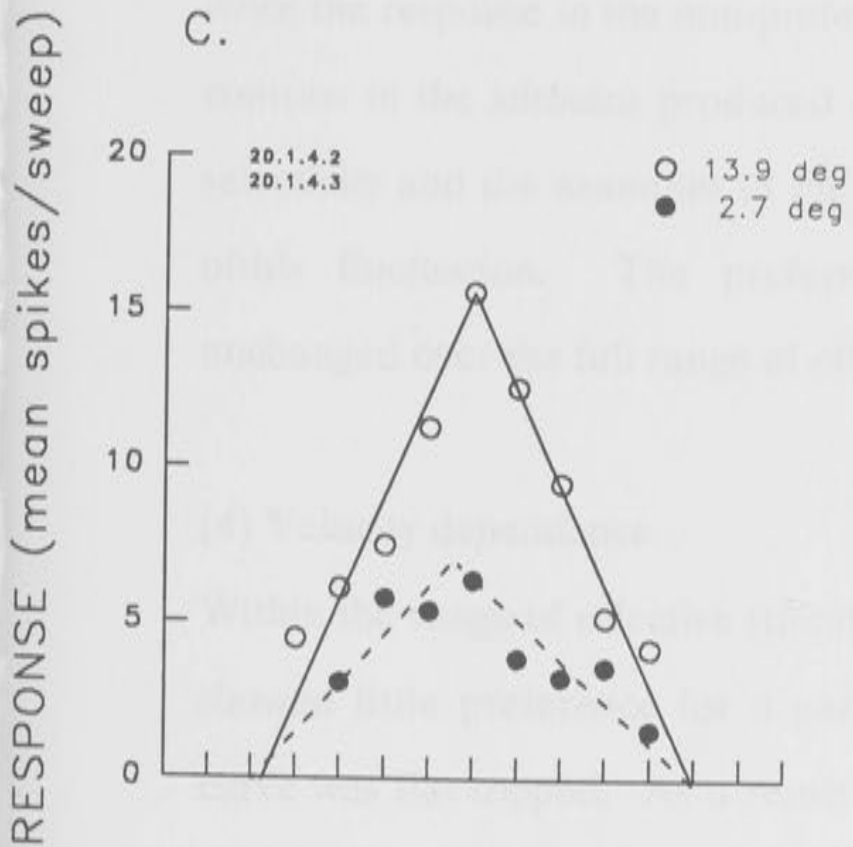
A.



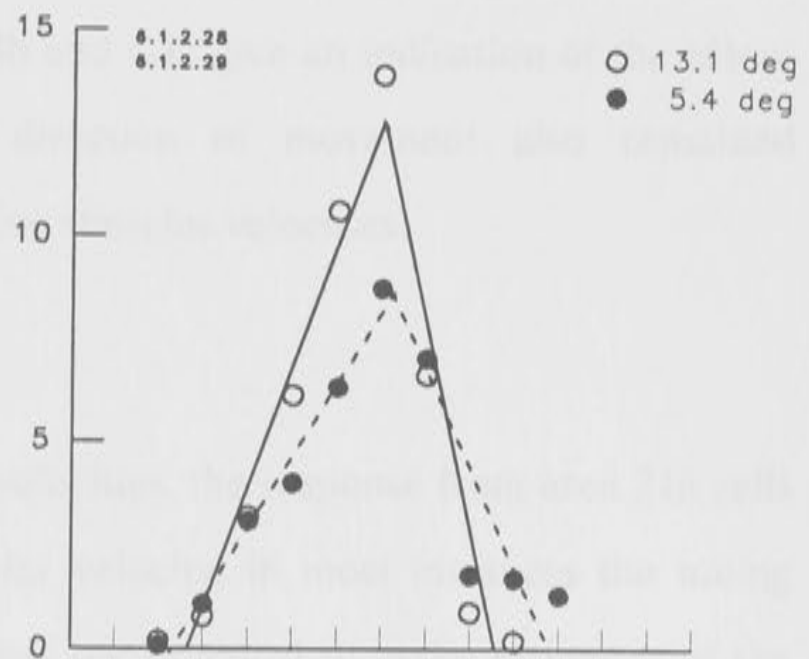
B.



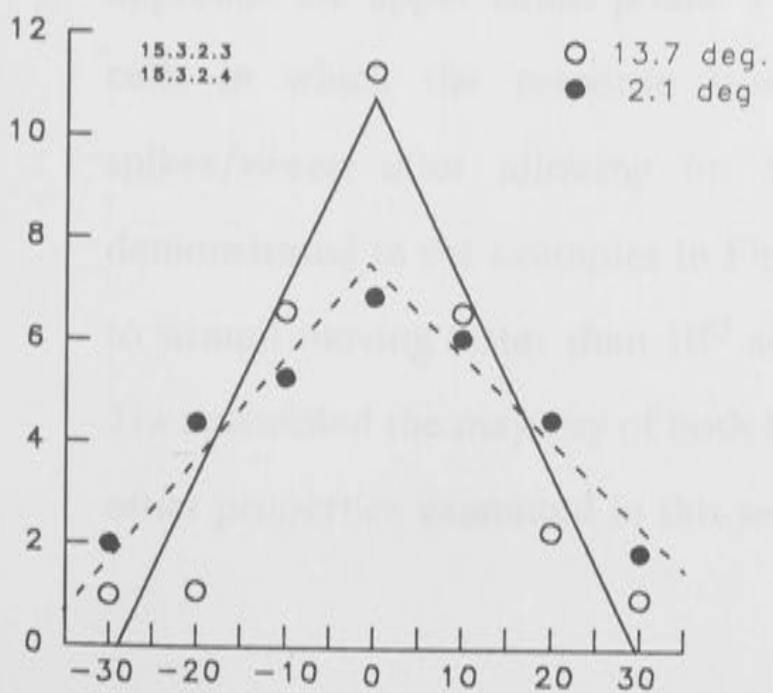
C.



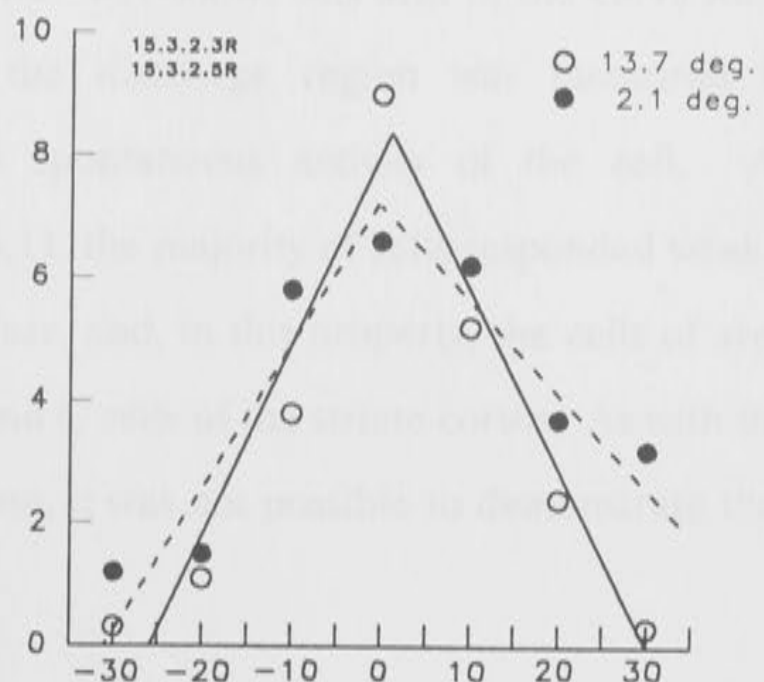
D.



E.



F.



ORIENTATION OF STIMULUS (deg)

(3) Direction selectivity

Direction selectivity in the visual responses of cells in the striate cortex has been attributed to the unilateral spread of inhibition (Goodwin and Henry, 1975; Goodwin et al., 1975). Similar inhibitory effects were not evident in the responses of cells in area 21a. All the cells in the present sample were bidirectional and no examples were found where there was a total absence of response in the non-preferred direction. Figure 4.10 shows four typical examples of average response histograms to a bar of light passed forward and backward across the cells' receptive field. In most cases the response in the preferred direction was less than twice the response in the non-preferred direction. Changes in the polarity of light contrast in the stimulus produced only minor changes in the degree of direction selectivity and the examples in Fig. 4.4b and 4.4c give an indication of the extent of this fluctuation. The preferred direction of movement also remained unchanged over the full range of effective stimulus velocities.

(4) Velocity dependence

Within the range of effective stimulus velocities, the response from area 21a cells showed little preference for a particular velocity; in most instances the tuning curve was flat topped. As a result it was not practical to make estimates of the preferred velocity in the present sample of cells. Typically, the velocity tuning curve showed its greatest rate of change as the stimulus velocity was increased to approach the upper cutoff point. Figure 4.11 shows this arm of the curve for 4 cells in which the response from the discharge region was measured in spikes/sweep after allowing for the spontaneous activity of the cell. As demonstrated in the examples in Fig. 4.11, the majority of cells responded weakly to stimuli moving faster than 10^0 arc/sec. and, in this property, the cells of area 21a resembled the majority of both S and C cells of the striate cortex. As with the other properties examined in this section, it was not possible to demonstrate that

FIGURE 4.10

Four average response histograms indicating the bidirectional character of a typical area 21a cell when a stimulus of preferred orientation is passed forward and backward in the visual field . The response in the preferred direction was usually less than twice the response in the non-preferred direction: a feature commonly found in area 21a cells.

RESPONSE (spikes/second)

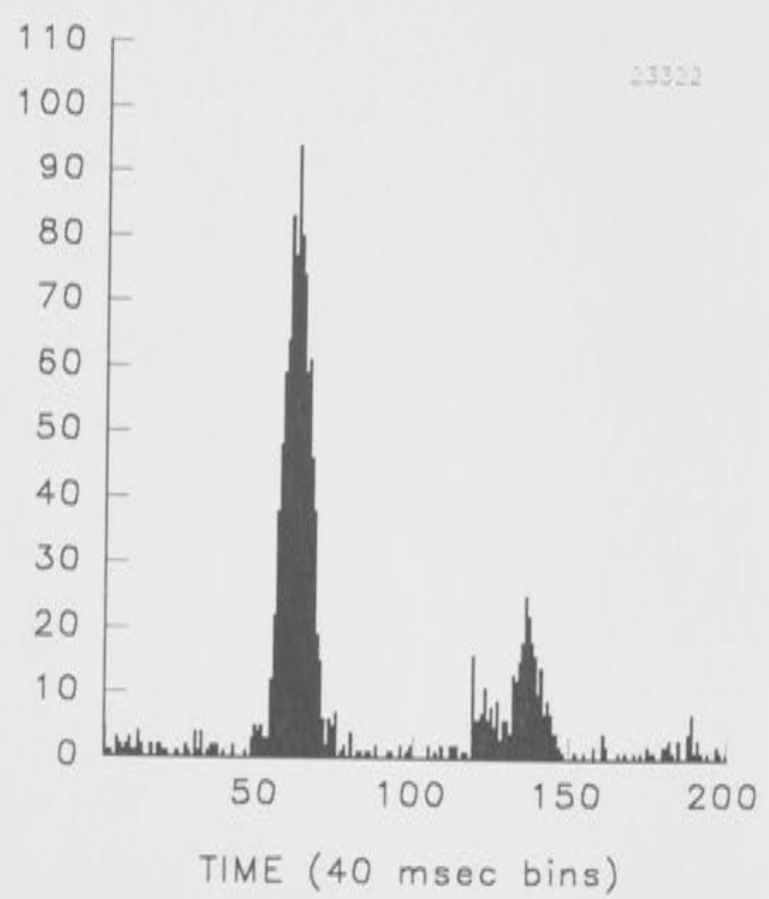
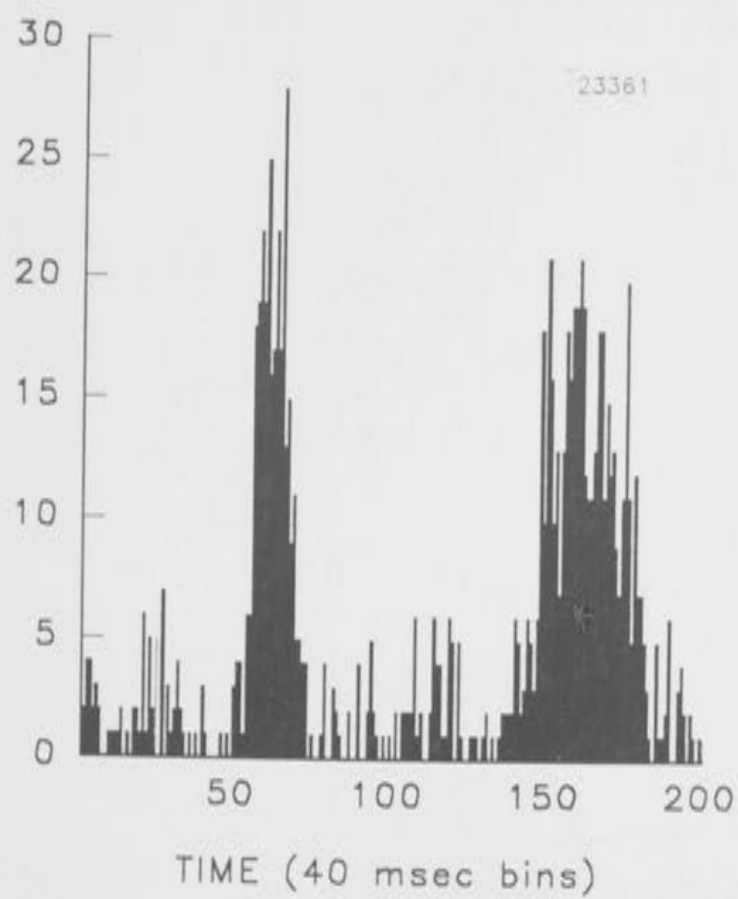
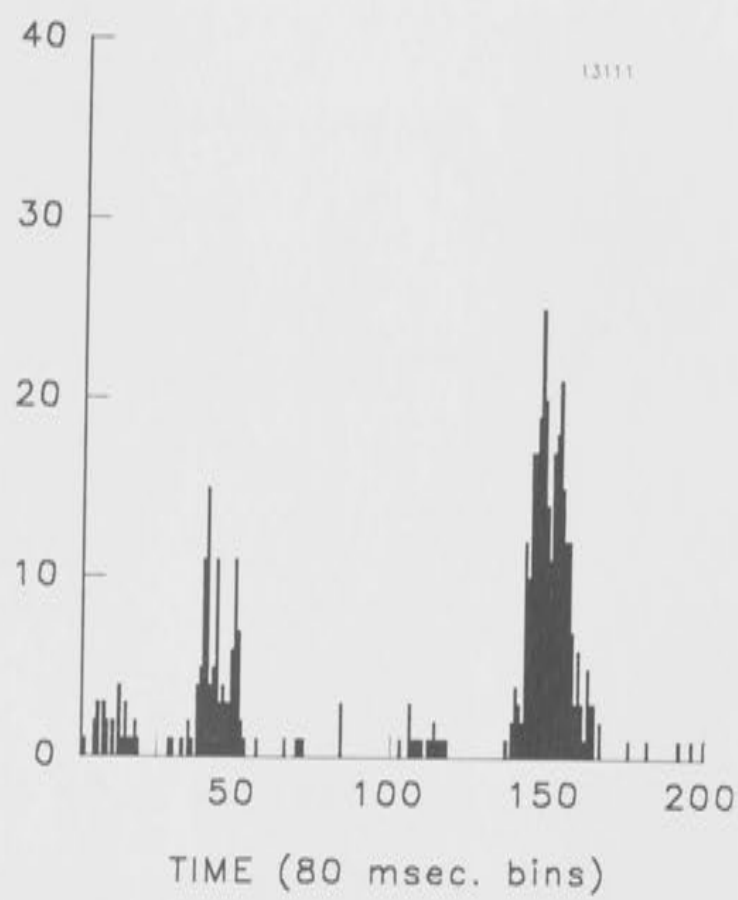
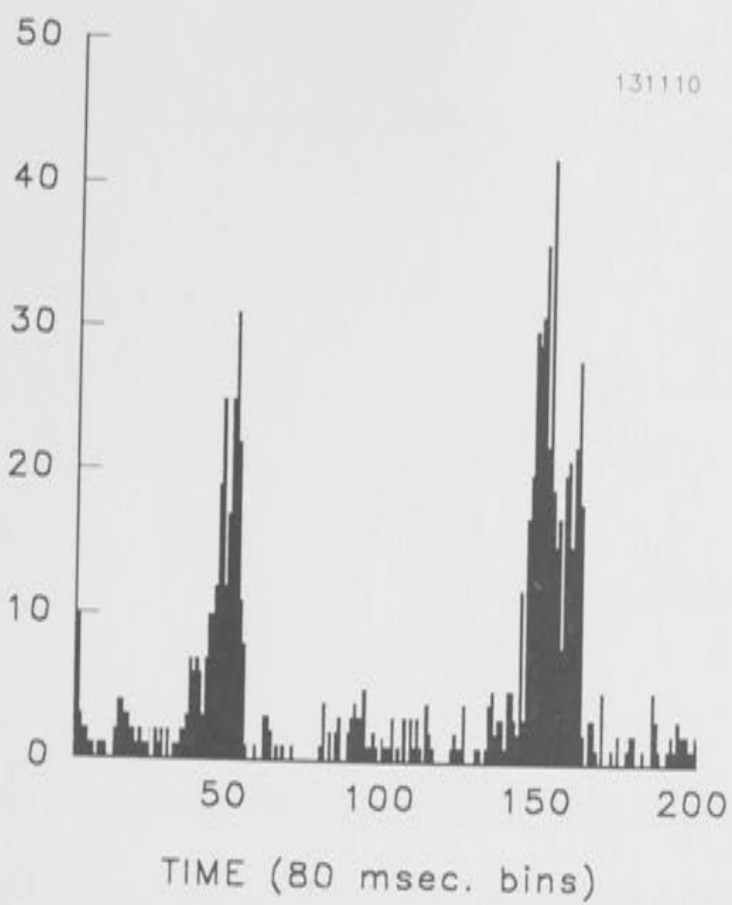
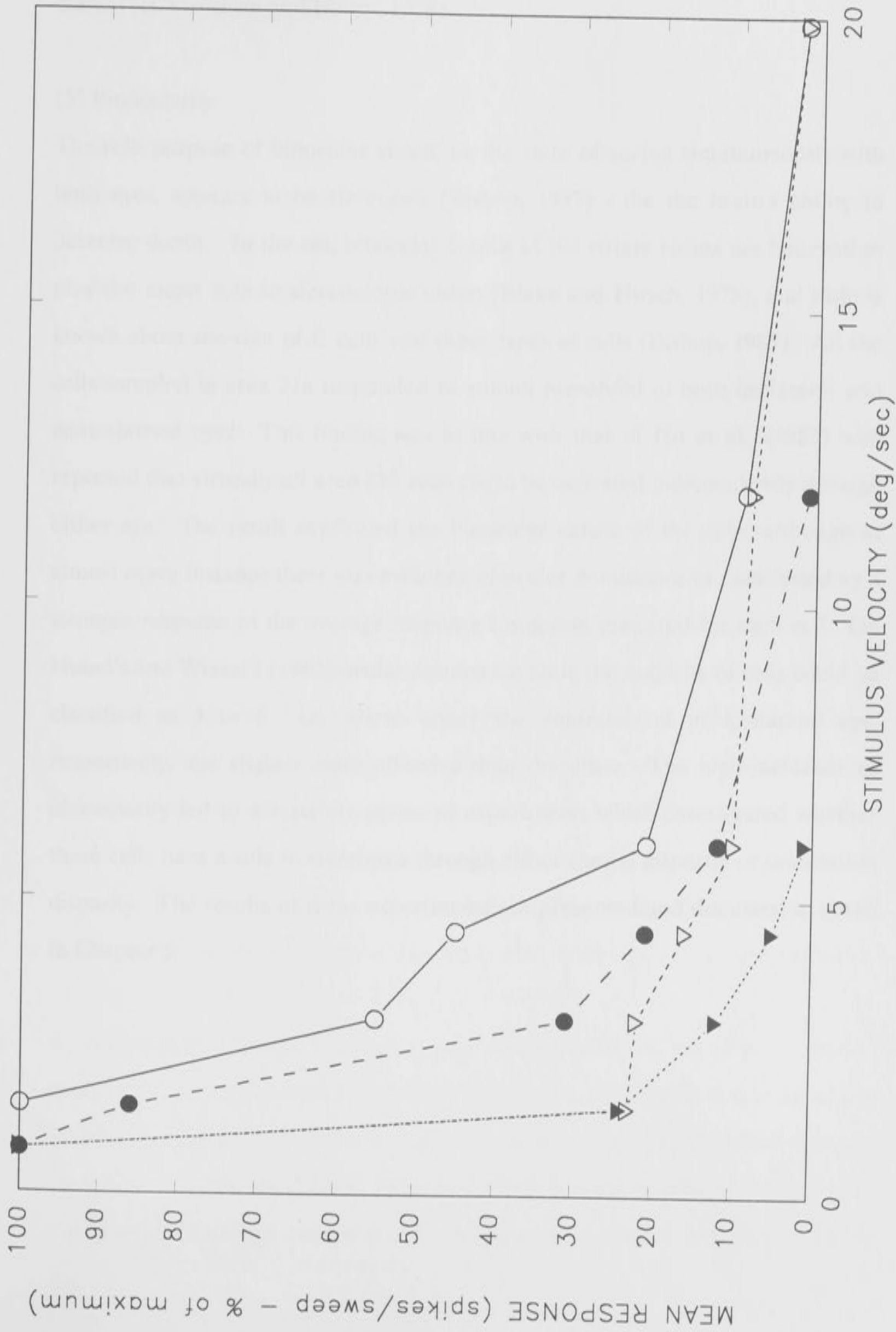


FIGURE 4.11

Upper arm of velocity tuning curves for 4 typical cells of area 21a demonstrating each cell's response to a stimulus of preferred orientation traversing the receptive field at a range of velocities. The response, measured in spikes/sweep, excludes spontaneous activity. All the cells respond preferentially to slow stimulus velocities (less than 2.0 deg.arc/sec) and that there is little response at velocities exceeding 10 deg.arc/sec.



local inhibition played a part in diminishing the response to the faster moving stimuli (cf. Goodwin and Henry, 1978)

(5) Binocularity

The sole purpose of binocular vision, or the state of seeing simultaneously with both eyes, appears to be stereopsis (Bishop, 1987) - the the brain's ability to perceive depth. In the cat, binocular S cells of the striate cortex are believed to play the major role in stereoscopic vision (Blake and Hirsch, 1975), and little is known about the role of C cells and other types of cells (Bishop, 1987). All the cells sampled in area 21a responded to stimuli presented to both ipsilateral and contralateral eyes. This finding was in line with that of Ho et al., (1982) who reported that virtually all area 21a cells could be activated independently through either eye. The result confirmed the binocular nature of the cells, although in almost every instance there was evidence of ocular dominance as manifested by a stronger response in the average response histogram prepared for each cell. On Hubel's and Wiesel's (1962) ocular dominance scale the majority of cells could be classified as 3 or 5 i.e. where either the contralateral or ipsilateral eye, respectively, was slightly more effective than the other. The high incidence of binocularity led to a separate series of experiments which investigated whether these cells have a role in stereopsis through either spatial disparity or orientation disparity. The results of these experiments are presented and discussed in detail in Chapter 5.

DISCUSSION

A number of salient features emerge from the sampling of the receptive fields of neurons in area 21a. In the first place there is a notable consistency in the type of receptive field recorded for every cell and the response properties are so similar that all cells can be placed in a single functional class. Dreher (1986) noted that about 90% of cells in area 21a were C type.

In their early recordings from the cat's striate cortex, Hubel and Wiesel (1962) defined S or simple cells on the basis of 4 characteristics. The cell's receptive field needed to exhibit spatially separate ON/OFF regions, summation within each region, antagonism between ON and OFF sub-regions and response properties that could be predicted from receptive fields maps. Complex or C cells were defined as cells which failed to show these characteristics. In a revised nomenclature for visual cortical cells, Henry (1977) considered that the receptive fields of C cells have the following key properties: (1) a uniform receptive field in which mixed ON/OFF discharges may be evoked at all points; (2) the ON/OFF discharges are close to maximum strength within an extended area of the receptive field. For a moving edge or bar stimulus also the firing rate rises and falls but remains close to maximum strength over much of the discharge region.

All cells recorded in area 21a have receptive fields which appear to be composed of a single discharge or excitatory region and there are few signs of accompanying inhibitory regions. The excitatory region produces composite ON/OFF firing in response to stationary flashing bars and, with moving stimuli, the same area produces overlapping responses to light and dark edges and bars. Other

properties common to the excitatory region include a binocular drive, little evidence of direction selectivity, an indication of summation which fell short of the linear expectation and extremely sharp tuning for a particular stimulus orientation. The acuteness of orientation tuning seemed, in almost every case, to be independent of the length of the test stimulus. The absence of direction selectivity and a preference for relatively slow moving stimuli argue against the involvement of area 21a in movement detection. On the other hand, the area may be concerned with some type of pattern recognition.

On the basis of my findings, it is possible to make some predictions on the location and type of cells in other visual areas that act as precursors to the cells in area 21a. A number of published axonal studies (Symonds et al., 1981; Lee et al., 1982; Raczkowski and Rosenquist, 1983; Symonds and Rosenquist, 1984a,b; Rosenquist, 1985; Dreher, 1986) as well as those performed in this laboratory (see Chapters 1 and 2), indicate that the afferents to area 21a arise from other cortical areas and from several regions of the diencephalon. It will be recalled that the latter arise from the C layers of the LGNd, the lateral segment of the lateral posterior nucleus (LPl) and from the pulvinar, whilst projections from other parts of the visual cortex emanate from cells in areas 7, 17, 18, 19, 20a and b, 21b, PMLS, PLLS AND DLS.

There are good reasons, however, for considering the cells in area 17 as the prime precursors, responsible for the characteristic responses of area 21a neurons. As has been indicated, the densest input to area 21a arises from area 17 although substantial inputs also originate in areas 18 and 19. Area 17, however, is the only cortical area to receive X-type geniculate inputs which have high spatial resolution and are thought to play a major part in form or object vision (Kaas, 1986). Turning to projections from the thalamus, tracer experiments indicated

that those originating from the pulvinar and the small cell C layer of the LGNd are relatively weak and therefore unlikely to have a major influence on area 21a. Although the projection from the LPI appeared to be strong, its major connections are with cortical areas especially area 17.

There are also good physiological reasons for believing that area 17 is likely to provide the most influential input to area 21a. In their response patterns and receptive field dimensions, the cells of area 21a more closely resemble those of the neurons of area 17 than those of other sources. Moreover, there are features of the response patterns of cells in the sources outside the striate cortex which seem incompatible with the responses recorded in the cells of area 21a. For instance, a relatively large proportion of neurons in area 19 display the hypercomplex or H property (i.e. end zone inhibition beyond one or both of the lateral borders of the discharge centre) which is not present in area 21a cells; and likewise the cells of area 18 prefer much faster stimuli than the cells of area 21a. Of the denser projections from the brain stem, the cells in the LPI and the pulvinar have larger receptive fields (Mason, 1978, 1981; Benedek et al., 1983) than required for a precursor to the cells of area 21a. On the grounds of greater compatibility, therefore, there is justification for a comparison between the response properties of cells in area 21a and area 17.

A key question to emerge from these results is the one that asks which of the two cell types in area 17, the S (simple) or C (complex) cell, is the more likely to be the precursor to area 21a cells. At first glance the receptive fields of the cells in area 21a seem more like those of C than S cells. The uniformity of the receptive field in the disposition of regions of ON and OFF response, light and dark edge response and light and dark bar response is the same as that of C cells. Henry (1977) considered that only cells with a uniform receptive field and mixed

ON/OFF discharge should be classified as C cells. The absence of linear summation and the preference for stimulus velocities of less than 10^0 arc/sec in area 21a cells is also consistent with the C cell response pattern. Although a cell's level of spontaneous activity should not be regarded as a definitive characteristic for distinguishing different classes of cell (Henry, 1977), S cells usually have little or no spontaneous discharge (Bishop and Henry, 1972). In contrast, area 21a cells usually displayed a consistent, although sometimes low, level of spontaneous activity. This suggests that their precursors are not S cells. On the other hand, the mean size of the receptive fields (mean width = 2.1^0 arc) is smaller than that expected from a C cell input. In addition, the average cell of area 21a is more sharply tuned for orientation than the C cell of the striate cortex. Both these distinctions, however, could result from the existence of a threshold barrier in the cells of area 21a - a possibility which is taken-up later.

Most of what has been said of C cells, however, could be applied with equal force to S cells. A single response region at light ON/OFF, to light/dark edges and to light/dark bars could occur in area 21a receptive fields with the overlapping and appropriate positioning of a number of S cell receptive fields. The lack of linearity is to be expected in higher cortical areas; the preference for stimulus velocities of less than 10^0 arc/sec and the dimensions of the receptive field could be consistent with an S cell ancestry whilst the sharpness of orientation tuning are more in keeping with an S than a C cell.

Failure to find evidence of significant inhibitory inputs in the receptive fields of area 21a cells is a characteristic feature. On the basis of extracellular recording, it has been assumed that the interaction of excitatory and inhibitory inputs determines the structure of a cell's receptive field. It has been proposed that excitation of cortical neurons is determined solely by thalamic afferents (Hubel

and Wiesel, 1962; Heggelund, 1981; Ferster, 1987,1988). In a model where this occurs, receptive field properties, including directional selectivity and sharpness of orientation tuning, may arise as a result of the shaping of relatively unselective excitatory inputs from the thalamus by postsynaptic inhibitory processes at the level of the cortex. A somewhat different model, however, was put forward recently by Douglas and Martin (1991) who suggest that the thalamic afferents provide a relatively small initial excitatory signal which can be easily controlled by inhibition. Since thalamic inputs can be amplified greatly by the considerable divergence in the intracortical connections of excitatory neurons (Gilbert and Wiesel, 1979; Martin and Whitteridge, 1984; Kisvardy et al., 1986; Gabbot et al, 1987), Douglas and Martin argue that it is connections from other cortical neurons (specifically spiny cells) that provide most excitatory input to a single cortical cell rather than an increase in thalamic inputs. The maximum discharge of a population of spiny cells may then be determined by inhibitory inputs from a population of smooth cortical cells. In such a model, the shape of the tuning curves depend on the timing and numbers of receptive fields of spiny and smooth cells which are stimulated. This model was put forward to account for the earlier finding of Douglas, Martin and Whitteridge, (1991) that the degree of inhibition required to modify the excitatory drive was not apparent from intracellular recording and could not be accounted for as a result of an inhibitory "shunting" mechanism from synapses distant from the soma.

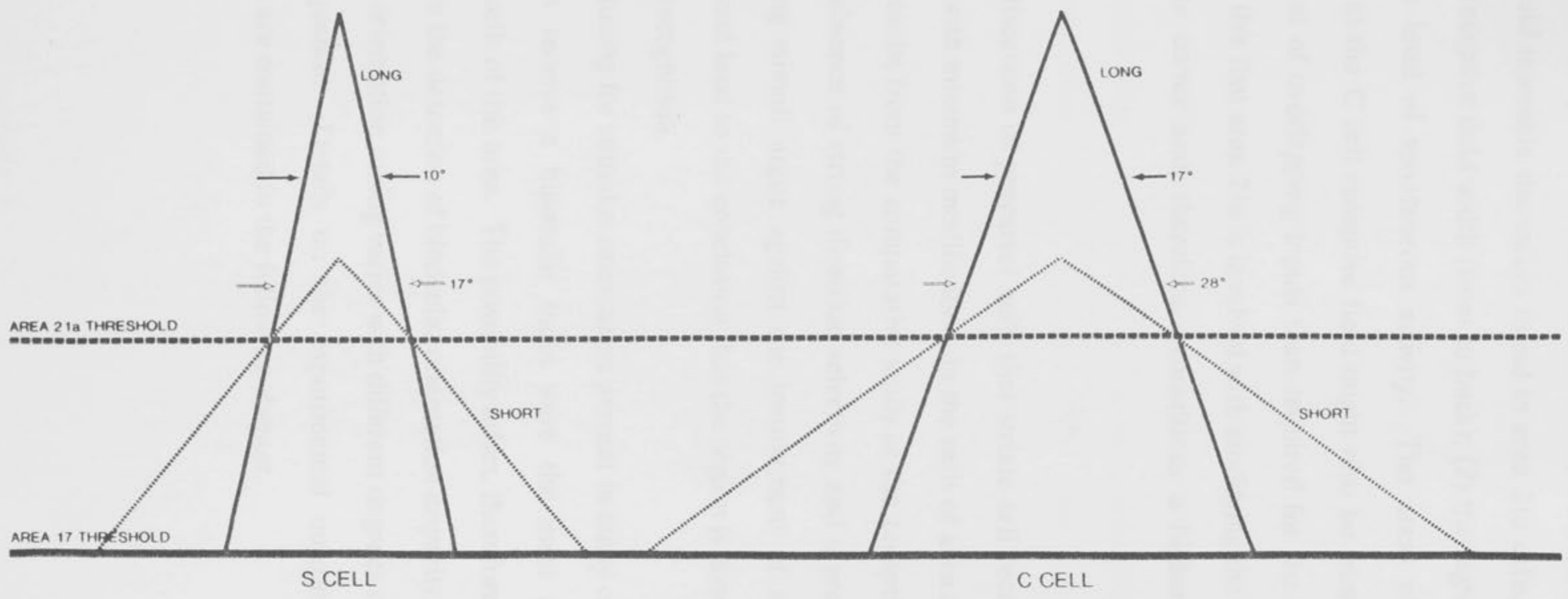
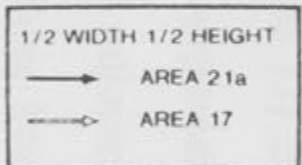
Whatever the source of inputs to area 21a, one of the more striking features of the striate S cell receptive field, which was absent from the response of the cells of area 21a, is the inhibitory regions that flank the primary borders of the excitatory discharge region (Bishop et al., 1973). The absence of inhibition does not rule out the possibility of an S cell parentage, however, since an area of active inhibition in a precursor cell may be converted to a non-responsive region in the

receptive field of the recipient cell. One way of tracing inhibition along a neural sequence is to follow the suppression it exerts on an excitatory response, since this effect is transferred from one cell to the next. In this way, the sharpening of the orientation tuning curve that occurs in S cells of the striate cortex when the bar stimulus is lengthened so that it extends into the inhibitory flank at non-optimal orientations, should be repeated in the successor cell in area 21a, for example. The present study shows, however, that the cells in area 21a fail to mimic the S cell tuning curves and instead, they display the same acuteness in orientation tuning curves for bar stimuli of different lengths. This would not preclude the existence of inputs coming from S cells, however, if there was present, in the cells of area 21a, a threshold barrier which acted to preclude lower, incoming firing rates. A schematic representation of S and C cell orientation tuning curves, adapted from Henry et al. (1974a), is shown in Fig. 4.12 where the threshold barrier is delineated by the dashed line. With this step, the difference in the acuteness of the curves for the bars of different length is effectively removed, since the half width at half height is the same for both curves.

The existence of a threshold barrier would receive additional support if it could be shown that the orientation tuning curves for bars of various length were sharper in area 21a cells than in their precursor cells. This possibility is demonstrated schematically in Fig. 4.12 by the reduction in half width at half height which results when going from the area 17 threshold level to that of area 21a for both S and C cells. The similar width in the orientation tuning curves which was found for long and short bars for the cells in area 21a is consistent, therefore, with either an S or a C cell input. The degree of sharpening in area 21a cells (mean = 17.5°), however, is less than predicted from an S cell (10° in Fig. 4.12) but is closer to that expected when inputs from C cells meet a threshold barrier and the half width at half height changes from 28° to 17° as shown in Fig.

FIGURE 4.12

Schematic representations of orientation tuning curves for long and short bars showing how a threshold model can reconcile the patterns recorded in area 21a cells with those of S and C cells in the striate cortex. With the threshold set for striate neurons (heavy line) the tuning curve for the shorter bar is broader than that for the long bar in both the S and the C cell. When the threshold for area 21a applies (dotted line) the tuning curves for both bars are equally sharp. In passing from the area 17 to the area 21a threshold the mean $1/2$ width are $1/2$ height changes from 17° (open arrow) to 10° (dark arrow) for the S cell and from 28° to 17° for the C cell. The C cell modification, therefore, is more in keeping with the tuning found in area 21a cells.



4.12. Other receptive field parameters of the cell which, on passing through a threshold barrier would resemble the values found in area 21a cells, include (1) the mean values for receptive field width (front to back); (2) the highest effective velocity and (3) the level of spontaneous activity. The areas of composite ON/OFF responses in the C cell receptive field might also be transposed with less spatial adjustment of overlapping inputs than required for the S cell. We might conclude from this that area 21a is involved with modifying the inputs from C cells of the striate cortex and, therefore, constitutes a higher level in a processing hierarchy.

It is implicit in this discussion of precursor cells that striate cell receptive fields are being replicated, with minimum modification, in the cells of area 21a. This is in keeping with the results from the comparative study of the receptive fields of the two areas. The absence of strong direction selectivity and a preference for relatively slow moving stimuli argue against the involvement of area 21a in movement detection and lead to the conclusion that the region is concerned with some type of pattern recognition.

The extremely sharp tuning for stimulus orientation present in many cells and the finding that all cells receive a binocular input were the most outstanding characteristics of the cells of this area. The possibility arises, therefore, that area 21a may be involved in the detection of binocular orientation disparity, and in this role the way in which orientation tuning varies with different degrees of binocular overlap is of consequence. Details on the experimental outcome of such binocular interactions are contained in the following chapter.

Conclusion

In summary, several tentative conclusions on the role of area 21a may be drawn. Despite the fact that the receptive fields of area 21a cells are moderately large, their central location and extent of overlap suggests that the area plays an important role in visual acuity. Confirmation that the cells are poor motion detectors came from their failure to respond well to fast moving stimuli. Conversely, the cells' strong responses to stationary flashing light bars and to slowly moving bars and edges pointed to the likelihood that they function as feature detectors - a possibility which, in many instances, was heightened by the cells' acuteness of orientation tuning. Failure to find evidence of sideband inhibition, however, implied that the cells' sharp orientation tuning was attributable to some other cause, possibly a threshold barrier which excludes low firing rates. The presence of such a barrier in area 21a could enhance the incoming edge orientation signals from the striate cortex in such a way that those arising from C cells were appropriately sharpened. In this way the pattern of edge orientations reaching area 21a may be "refined" or modified prior to undergoing further processing.

APPENDIX
MANN-WHITNEY TEST

Orientation Tuning
Half Widths at Half Height

<u>Short Bars(deg)</u>	<u>Rank</u>	<u>Long Bars(deg)</u>	<u>Rank</u>
17	10	16	9
12	3	11	1
14	7	12	3
12	3	13	5.5
13	<u>5.5</u>	15	8
	$R_1 = 28.5$		

$$U = N_1 N_2 + \frac{N_1 (N_1 + 1)}{2} - R_1$$

$$U = 5 \times 5 + \frac{5(6)}{2} - 28.5$$

$$U = 11.5$$

$$U_1 = N_1 N_2 - U$$

$$U_1 = 5 \times 5 - 11.5$$

$$U_1 = 13.5$$

As the smaller observed U (11.5) is greater than the critical value of U for 5 per cent significance (2), it cannot be concluded that there is a significant difference between the two samples i.e there is no reason to believe that the two groups were drawn from separate populations.

INTRODUCTION.

Cats, in common with monkeys and humans, have forward viewing eyes and the cells in their visual cortex generally have binocular receptive fields that overlap each other. Unlike animals with eyes positioned at the sides of the head that provide an almost 360° view of the world, they do not have the advantages of panoramic vision.

CHAPTER 5

The horizontal separation of the eyes in such species produces images on each retina that have small retinal disparities. If the visual cortex these disparities are

THE DETECTION OF BINOCULAR ORIENTATION DISPARITY

of stereoscopic vision which encompasses these species are the loss of panoramic vision. Blake and Hirsch (1975) demonstrated that a normal cat using binocular vision can detect a spatial disparity of less than 4 minutes of arc which represents an order of magnitude better than the smallest depth detectable under monocular viewing conditions.

Disparities between the two retinal images also exist with respect to orientation and these commonly arise when a viewer looks down at an object, for instance when averting the eyes to read or to view the ground ahead (Nalwa et al., 1977). If the eyes are at their normal vertical angle retinal images that have considerable orientation differences must arise from an object that is close to the viewer and this therefore signal the proximity of the object (Matheson et al., 1972).

Despite the existence of orientation disparity in the two retinal images it is still possible to achieve binocular fusion. Since the time of Wheatstone (1838), however, there has been considerable debate as to whether fusion into a single image (a phenomenon termed cyclopia) was due to cycloversion of the eyes or to a central neural mechanism. In fact, both a central neural mechanism and a cycloversion response

INTRODUCTION.

Cats, in common with monkeys and humans, have forward viewing eyes and the cells in their visual cortex generally have binocular receptive fields that overlap each other. Unlike animals with eyes positioned at the sides of the head that provide an almost 360° view of the world, they do not have the advantages of panoramic vision. The horizontal separation of the eyes in such species produces images on each retina that have small spatial disparities. In the visual cortex these disparities are transposed to represent depths in visual space and it would seem that it is this faculty of stereoscopic vision which compensates these species for the loss of panoramic vision. Blake and Hirsch (1975) demonstrated that a normal cat using binocular vision can detect a spatial disparity of less than 4 minutes of arc which represents an order of magnitude better than the smallest depth detectable under monocular viewing conditions.

Disparities between the two retinal images also exist with respect to orientation and these commonly arise when a viewer looks down at an object, for instance when averting the eyes to read or to view the ground ahead (Nelson et al., 1977). If the eyes are at their normal torsional angle retinal images that have considerable orientation differences must arise from an object that is close to the viewer and might therefore signal the proximity of the object (Blakemore et al., 1972).

Despite the existence of orientation disparity in the two retinal images it is still possible to achieve binocular fusion. Since the time of Wheatstone (1838), however, there has been considerable debate as to whether fusion into a single image (a phenomenon termed cyclofusion) was due to cyclorotation of the eyes or to a central neural mechanism. In fact, both a central neural mechanism and a motor response

in the form of compensatory eye movements appear to play a part in cyclofusion. The critical factor seems to be the length of the object being viewed (Bishop, 1987). Kertesz and Jones (1970) attempted to measure torsional movements in humans but found that long lines in a horizontal plane with retinal images that differed by around 5° in orientation could be fused in the absence of any cyclorotation of the eyes. Crone (1971) took the view that if cyclofusion does occur as a result of rotational eye movements, their maximum amplitude is very small. In an experiment in which short lines with mean vertical orientation were presented tachistoscopically to subjects Braddick (cf Blakemore et al., 1972) found that retinal images which differed by up to 15° could be tolerated before the lines were seen double. In real life there are numerous examples where, for fusion to occur, impossible eye movements would have been required and this, according to Bishop (1978) points to the undoubted role of a central neural mechanism. In Bishop's (1987) view, a central component is the major factor in cyclofusion for lines subtending less than 10° and little cyclorotation of the eyes is involved. A motor component comes into consideration, however, for line stimuli greater than 10° in length and may be the major influence for line lengths of 50° .

The responses of most neurons in the cat's visual cortex are orientation specific and the majority are also binocularly activated (Hubel and Wiesel, 1962; 1968; Bishop et al., 1971). In many of these cells the preferred orientation is identical in the two eyes but, in a sizeable proportion, the two orientation tuning curves are laterally displaced from each other. These cells, by analogy with *spatial disparity* in *receptive field* position, may be said to exhibit a *disparity* in their *orientation tuning*. For cells of the striate cortex the difference in preferred orientation in the receptive fields of each eye may vary by as much as 15° (Blakemore et al., 1972; Nelson et al.,

1977). Based on the premise that any differences in the two images falling upon the retina (*image disparity*) might be utilised by the visual system to facilitate stereoscopic vision, Blakemore et al., (1972) investigated the proposition that retinal orientation disparity might provide a second cue for depth perception. Although Blakemore et al. reached the conclusion that binocular cells of the striate cortex can signal the presence of tilted objects about their horizontal axis, Nelson et al. (1977) found that the cells of the striate cortex are relatively poor discriminators of orientation disparity in the retinal image and were unlikely, therefore, to act as detectors of this feature.

As a result of an investigation of the neuronal response characteristics of area 21a which were described in Chapter 3, it was found that the cells were sharply tuned for orientation, were almost all binocularly driven and had receptive fields that were within 15° of the visual axes. These properties were generally in accordance with the findings of other investigators, and meet many of the requirements for an orientation disparity detector. As a result additional electrophysiological experiments were undertaken to discover if the cells of area 21a have a special role in the detection of orientation disparity.

To examine the nature of the binocular interactions in area 21a cells binocular tuning curves were prepared for spatial and orientation disparity and compared with the orientation tuning curves recorded for each eye separately and for both eyes together. For the disparity tuning curves the stimulus to one eye remained unchanged while that to the other eye was varied, as required, either in spatial position or in orientation. In order to ensure that neither eye produced a biased response, only binocular cells which could be placed in Hubel and Wiesel's (1962) eye dominance groups 3, 4 or 5 were included in the sample.

while the stimulus before the other eye was rotated through the range of orientations required for the tuning curve.

MATERIALS AND METHODS

Animal Preparation

Experiments were performed on six adult cats, each weighing between 2 and 5 kg. Surgical procedures and the basic animal preparation were identical to those described in Chapter 4. The optic disks and areas centralis were back projected onto a tangent screen with the aid of a Zeiss fundus camera and changes in the degree of cyclotorsion were measured by back projecting at least two "landmarks" (usually the junction of blood vessels) at regular intervals throughout the experiment. All orientation measurements were corrected to allow for induced cyclotorsion.

Recording Procedures

Extracellular single unit recordings, made with tungsten-in-glass microelectrodes with an impedance of 2-10 M Ω . The visual stimuli comprised moving light or dark bars presented, under computer control, in 24cm x 24cm raster on an oscilloscope where the orientations of the bars were randomly varied and interleaved to produce a tuning curve in a single experimental run. In addition, each eye could be presented simultaneously with bars of different orientations so that disparity tuning curves were produced by keeping the stimulus before one eye fixed at the preferred orientation

while the stimulus before the other eye was rotated through the range of orientations required for the tuning curve.

RESULTS

Sample

Results were obtained from two separate experiments in area 21a, one in which monocular response characteristics were recorded from 115 cells, a second where both monocular and binocular orientation and disparity tuning curves were prepared for 50 cells. All cells in the sample were located within 2° of the visual axis and were binocular. Every cell tested responded with consistent ON/OFF firing from all points in their receptive fields.

Spatial Disparity

Four cells showed signs of being sensitive to spatial disparity. When stimulated binocularly the combined responses from the two eyes were seldom influenced by binocular enhancement or inhibition although these features were present in several 50% of the sample.

Monocular Orientation Tuning Curves

(1) Preferred Orientation

Most of the 115 cells tested monocularly were sharply tuned for stimulus orientation and the most favored orientations were vertical (in 21% of sample) and horizontal (34%). A distribution of preferred orientations is presented in Fig. 5.1. Pairs of

RESULTS

Sample

Results were collected from two separate experiments in area 21a; one in which monocular response characteristics were recorded from 115 cells; a second where both monocular and binocular orientation and disparity tuning curves were prepared for 50 cells. All cells in the sample were located within 15 degrees of the visual axes and were binocular. Every cell tested responded with composite ON/OFF firing from all points in their receptive fields.

Spatial Disparity

Few cells showed signs of being effective detectors of spatial disparity. When interacted binocularly the combined response from the two eyes was seldom influenced by binocular enhancement or inhibition although these features were present in around 5% of the sample.

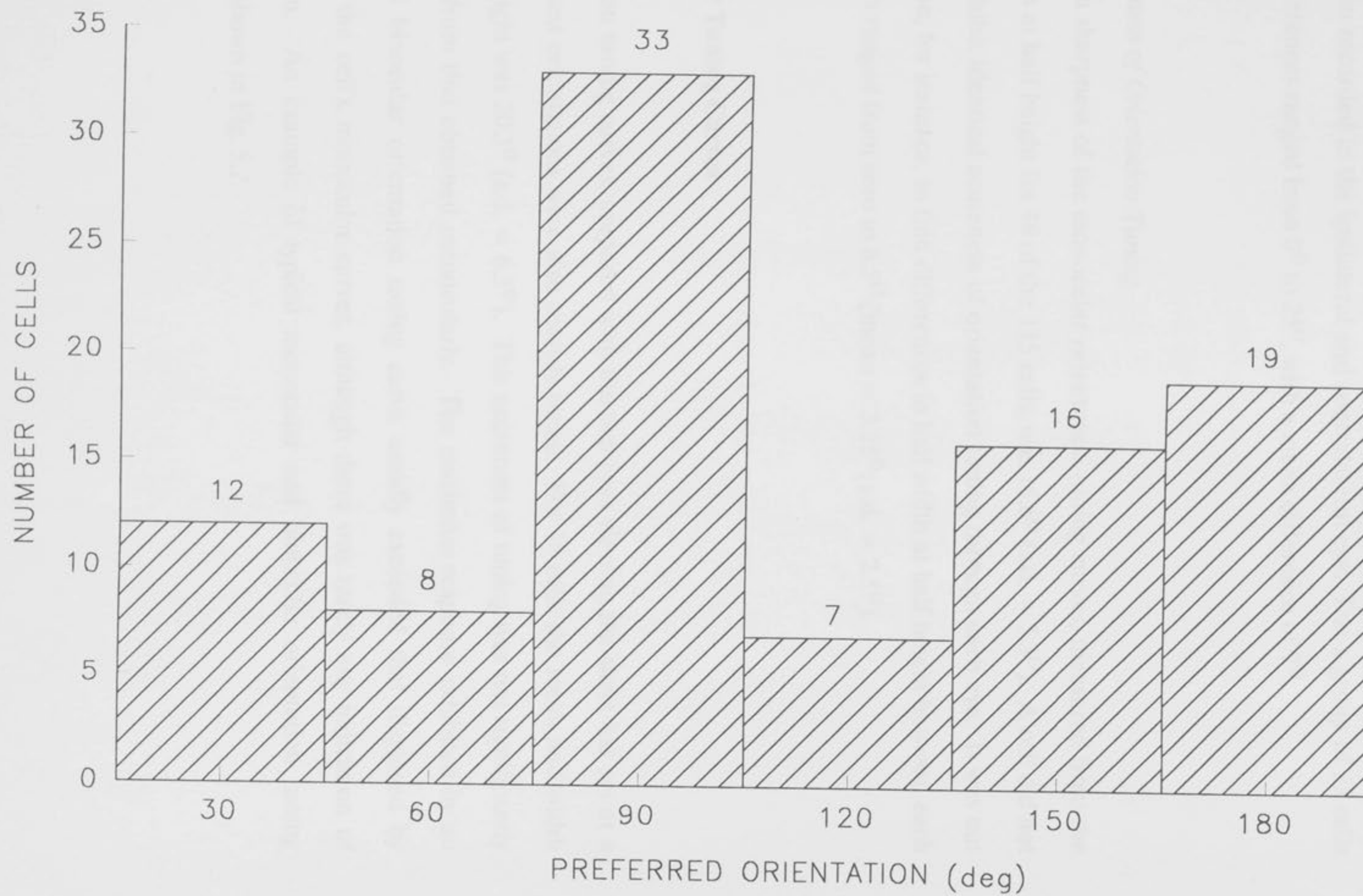
Monocular Orientation Tuning Curves

(1) Preferred Orientations

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FIGURE 5.1

A distribution histogram of preferred orientations for a sample of 95 cells in area 21a.



monocular orientation tuning curves were prepared in which the orientation was changed in 5° or 10° steps and, for most cells, there was a difference in the preferred orientation recorded in the ipsilateral and contralateral eye. For a group of 23 cells these differences ranged from 0° to 25° , with a mean difference of 7° .

(2) Sharpness of Orientation Tuning

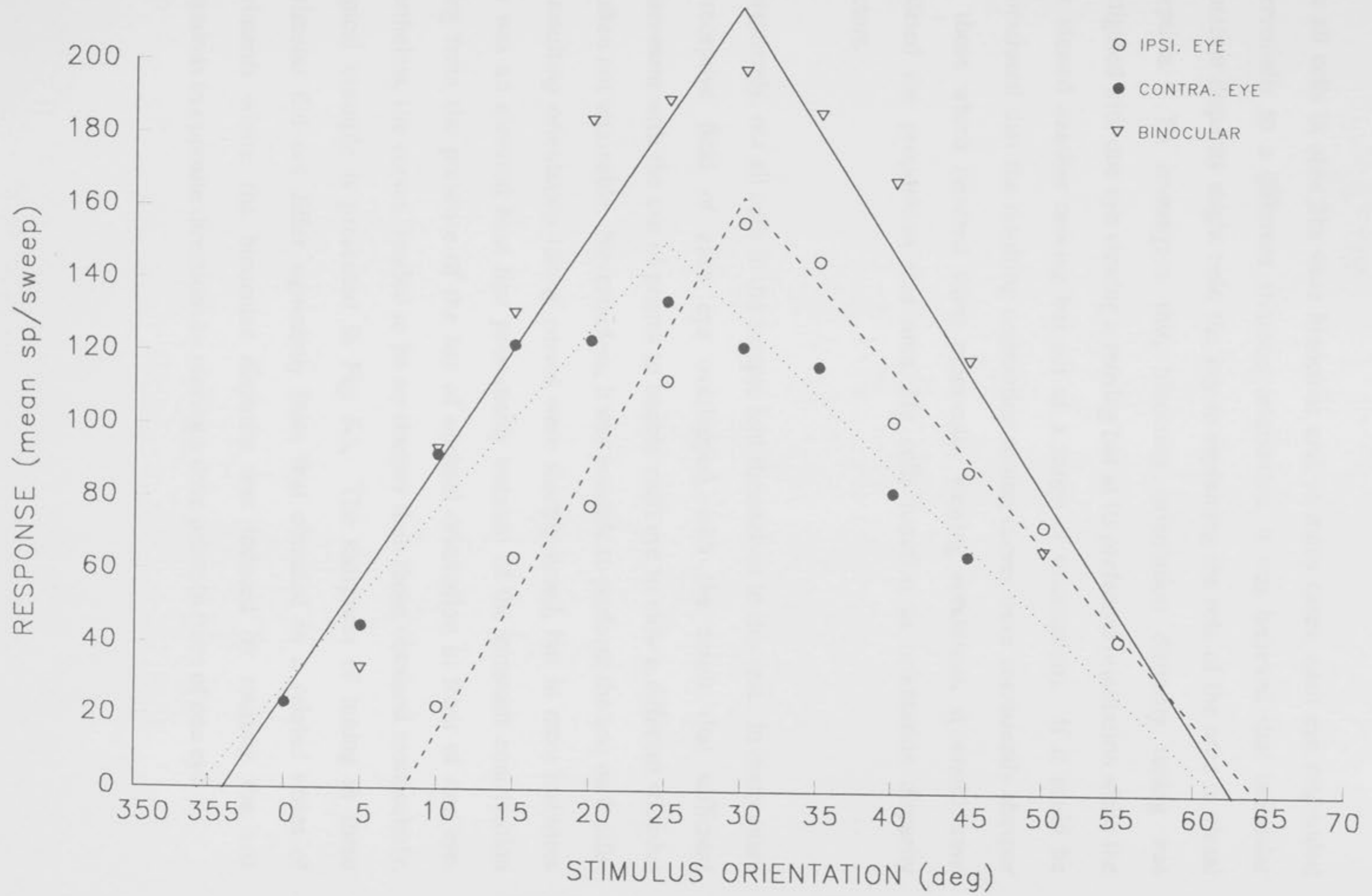
The mean sharpness of the monocular orientation tuning curves, measured from the half width at half height for 48 of the 115 cells, was 15.6° (s.d. = 5.3°). A cell did not always exhibit identical acuteness of orientation tuning through each eye. It was not uncommon, for instance, to find differences in half width at half height between each eye, which ranged from zero to 8.5° (mean = 3.25° ; s.d. = 2.5°).

Binocular Tuning Curves

Orientation tuning curves, prepared with the two eyes viewing the same bar set at a range of test orientations were also sharply tuned. For 16 cells the mean half width at half height was 20.3° (s.d. = 6.5°). This acuteness of tuning was not significantly different from that obtained monocularly. The maximum response exhibited by an individual binocular orientation tuning curve usually exceeded that displayed by either of the cell's monocular curves, although there was rarely any indication of facilitation. An example of typical monocular and binocular orientation tuning curves is shown in Fig. 5.2.

FIGURE 5.2

Regression lines fitted to monocular and binocular orientation tuning plots obtained from one cell. Note that the ipsilateral and contralateral eyes respond maximally at different orientations; and that there is little evidence of facilitation or inhibition in the binocular plot.



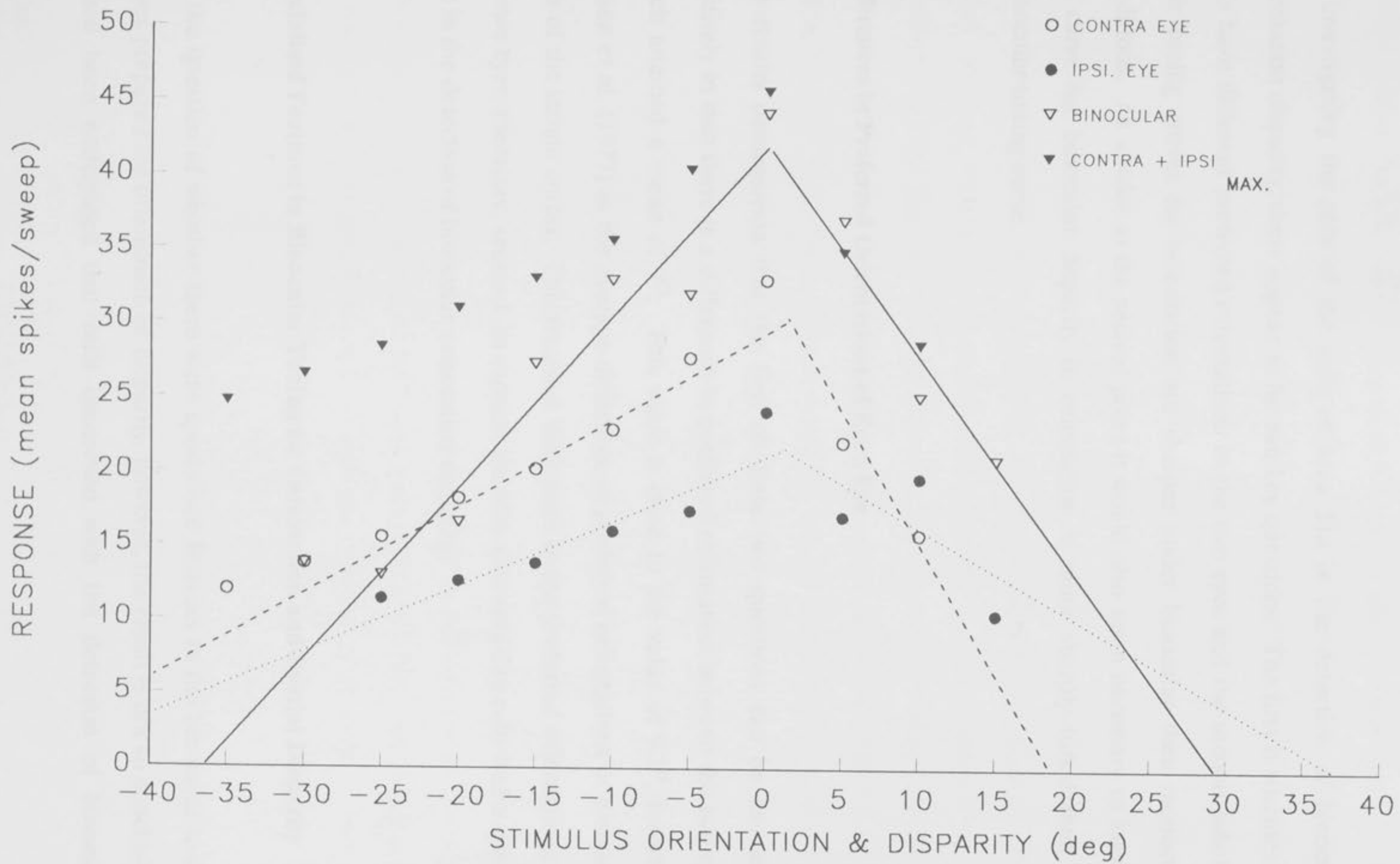
Binocular Orientation Disparity

Since all cells in area 21a were binocular and, in many cases, each eye responded preferentially to a different stimulus orientation, it was believed that binocular orientation disparity might hold the key to explaining the role of the area in visual perception. To investigate this, binocular orientation disparity tuning was investigated with one eye viewing a moving bar at its preferred orientation while the other viewed another moving bar set at a range of orientations. If it could be demonstrated that the resulting orientation tuning curves were consistently sharper than those which resulted from monocular viewing conditions, it would have supported the proposition that area 21a cells function as orientation disparity detectors.

Unfortunately, not all cells in the sample lent themselves to this test. In many cases the receptive field of each eye overlapped, with the result that sufficient displacement with the use of prisms to enable each eye to view a different stimulus was often not attainable. Nevertheless, it was possible to perform the test on 8 cells. The resulting orientation tuning curves were sharply tuned, but in most instances there was an elevated base line presumably because of the constant contribution coming from the presence of the bar of optimal orientation in front of one eye. Nevertheless, the curves tended to be no sharper than those obtained monocularly. A typical example is presented in Fig. 5.3. The sharpness of tuning in these experiments did not differ significantly from that obtained in a related series of experiments where the binocular disparity was induced by rotating the test orientations in opposite directions by placing a dove prism in front of one eye.

FIGURE 5.3

Regression lines fitted to binocular orientation disparity tuning plots and to monocular and binocular orientation tuning plots obtained from the one cell. The binocular orientation disparity responses were obtained with the ipsilateral eye viewing a bar stimulus at its preferred orientation while the contralateral eye viewed another bar set at a range of orientations. Note that the binocular disparity curve is similar to the sum of the two monocular curves.



(For binocular response, the ipsi. eye stimulus was set at the eye's preferred orientation)

DISCUSSION

In investigating the role of the cells of area 21a in the detection of binocular orientation disparity, there appear to be two key questions. The first is whether the cells have different preferred orientations in the two eyes and the second, whether their tuning curves for orientation are sharper under binocular than monocular conditions. As a rider to the second point it would also seem necessary to know if the curve for binocular disparity in orientation is more sharply tuned than the monocular tuning curve.

Differences in Preferred Orientations of Each Eye

The results demonstrate that the first of these two questions can be answered positively in that there is a difference in preferred orientation between the two eyes which attained a mean of 7° . This value is close to the value of 9.2° , found by Nelson et al. (1977) as the average difference in preferred orientations in binocular cells of the striate cortex. This frequent difference in the preferred orientations for the two eyes, therefore, seemed to support the idea that area 21a cells had a special role in the detection of binocular orientation disparity.

Specialised Features in Binocular Tuning for Orientation and Spatial Disparity

On the question of whether there were specialised features in the binocular tuning curves prepared for orientation or disparity, however, the result is not so conclusive. It had been anticipated that cells concerned with the detection of binocular

differences in orientation would display sharper tuning curves in going from monocular to binocular viewing conditions. However, the results generally indicated that the cells of area 21a do not possess an orientation-sensitive mechanisms which make binocular orientation tuning responses sharper than would be expected to result from monocular orientation mechanisms. The present series of experiments, therefore, failed to add support to the concept that area 21a cells operate as specialised binocular detectors.

Nor was the requirement of binocular superiority met in the preparation of *disparity* tuning curves which, in effect, are tuning curves of orientation differences. Once again, however, orientation disparity discrimination under binocular viewing conditions was not significantly sharper than when orientation tuning curves were prepared monocularly. These results do not give conclusive support to the proposition that area 21a cells have a unique role in the detection of disparity. Nevertheless, the sharpness of their orientation tuning and the high incidence of binocularity might still provide an effective base for such detection.

Blakemore et al. (1972) has pointed-out that cells with different preferred orientations in the two eyes may have other functions than signalling the rotation of objects in depth. For instance, they might be used to adjust the torsional angle of the eyes. In a situation where one eye rotates from its correct torsional position, cells with appropriately dissimilar preferred orientations will be stimulated and this information might be used by the cat to adjust the torsional position of its eyes and so maximise the activity of its cortical cells.

Nelson et al. (1977) concluded that the mean optimal stimulus orientation disparity for striate cells of 9.2° was attributable to ocular cyclorotation resulting from initial anaesthesia and paralysis i.e. assuming an incyclorotation for each eye averaging

4.6°. On the other hand Cooper and Pettigrew (1979) confirmed an earlier observation of Sanderson (1972) that the medial borders of the two visual hemifields are both vertical and parallel in the anaesthetised and paralysed cat, suggesting that rolling of the eyes had not occurred. These apparently conflicting results may be resolved if it is assumed that in the normal unanaesthetised state the medial borders of the cat's two hemifields are not parallel but crossed or out-torted. Under anaesthesia, however, they become vertical and parallel as a consequence of incyclorotation of the eyes.

Conclusion

The conclusion from this study, therefore, is that the cells of area 21a are not as effective as the S (simple) cells of the striate cortex in the detection of spatial disparity and, as well, they do not appear to add to the contribution of striate neurons in the processing of orientation disparity. The question that now requires attention is whether the striate neurons, and by analogy the area 21a cells, are as ineffective in the detection of binocular orientation disparity as previously proposed (Nelson et al, 1977). Failure to find that the cells of area 21a have a special role in the detection of orientation disparity does not invalidate a possible role for the area in depth perception. It should be emphasised that the size of the sample was small and further testing would need to be performed before any definite conclusion can be drawn.

INTRODUCTION

The analysis of the responses of neurons located in well interconnected cortical areas which are thought to represent sequential levels of information processing can provide an insight into the signal flow in the visual system. In the cat, however, such an analysis is difficult because of the lack of a clear-cut hierarchy of visual areas. Nevertheless, the present experiments described in Chapter 1, together with the results of several earlier investigations (Strydom et al., 1981; 1983; Draker, 1986) do not doubt on the suggestion that area 21a is the recipient of a strong retino-thalamic pathway, suggest that area 21a receives its major input from area 17 and perhaps also 18. While it might have been possible to confirm this by attempting to record from area 21a after bilateral ablation of the primary visual cortex, a more sophisticated alternative presented itself in the form of cortical deactivation by cooling.

THE EFFECT ON AREA 21A FOLLOWING COOLING OF THE PRIMARY VISUAL CORTEX

The controlled cooling of local areas in the cortex provides an elegant method which allows for the reversible blockade of electrical activity of the neurons located in those areas and is particularly useful in the analysis of brain function. The earliest method of local cooling was developed in the last years of the century by Trendelenburg (cf. Brody, 1983) who used brass-cooling chambers placed on the brainstem, spinal cord and auditory cortex of rabbits, cats, dogs and monkeys. Since 1980 the thermoelectric Peltier cell has become the preferred method to control local

INTRODUCTION

The analysis of the responses of neurons located in two interconnected cortical areas which are thought to represent sequential levels of information processing can provide an insight into the organisation of the cortical network simply by revealing what was added, subtracted or altered at the higher level. In the cat, however, as has been indicated, a strictly hierarchical model of visual processing does not obtain, which makes it difficult to settle upon a pair of visual areas which are suitably interconnected. Nevertheless, the tracer experiments discussed in Chapter 3, together with the results of several earlier investigations (Symonds et al., 1981; Raczkowski and Rosenquist, 1983; Symonds and Rosenquist, 1984a,b; Rosenquist, 1985; Dreher, 1986) demonstrated the presence of robust connections between area 21a and the primary visual cortex. These results together with the findings of Chapter 1 which cast doubt on the suggestion that area 21a is the recipient of a strong retino-thalamic pathway, suggest that area 21a receives its major input from area 17 and perhaps area 18. Whilst it might have been possible to confirm this by attempting to record from area 21a after bilateral ablation of the primary visual cortex, a more sophisticated alternative presented itself in the form of cortical deactivation by cooling.

The controlled cooling of local areas in the cortex provides an elegant method which allows for the reversible blocking of electrical activity of the neurons located in those areas and is particularly useful in the analysis of brain function. The earliest method of local cooling was developed in the first years of this century by Trendelenberg (cf Brooks, 1983) who used brine-cooled chambers placed on the brainstem, spinal cord and sensorimotor cortex of rabbits, cats, dogs and monkeys. Since 1970 the thermoelectric Peltier cell has been the preferred method to control tissue

temperatures up to several millimeters from the implant. Peltier cells absorb or release heat at junctions of dissimilar metals during transfer of electric charge. The potential energy level of one conductor to the potential energy level of the other conductor (heat) is either absorbed or emitted to maintain conservation of energy (Hayward et al., 1965).

Animal Preparation

The major advantage of controlled cooling is that it permits repeated, brief, local, reversible dysfunctions to occur, without the neural reorganisation that often results from permanent lesions. Under these circumstances the response characteristics of a single neuron can be evaluated before, during and after several cooling trials. These data can then be used to make deductions concerning causal relations between neural function and other events at the cellular, organ or behavioural level (Brooks, 1983). The main limitation of the method is the imprecise knowledge of the extent of inactivated tissue. This arises from diverse effects of different temperatures at the cellular level (Brooks, 1983).

Controlled local cooling combined with extracellular recording from single units, therefore, offered a useful means for confirming the origin of the major cortical afferents to area 21a and for determining their functional contribution. It has already been pointed-out that all the cells of area 21a, from which electrophysiological recordings were made (Chapters 4 and 5), exhibited orientation selectivity and many of them were more acutely tuned for orientation than S cells of the striate cortex. These findings together with the results of anatomical investigations implied that the pathway from the primary visual cortex might be the sole connection responsible for conveying data that determines the response properties of area 21a, particularly orientation tuning. In order to test this hypothesis, recordings were made from cells in areas 17 and 21a before and after their input from the primary visual cortex in both hemispheres was selectively removed by reversible cooling. *Experiments were continued*

1mm below the cortical surface. Fig. 6.1 illustrates an anaesthetized cat in the stereotaxic device and the relative positions of the cooling device, thermocouples and recording microelectrode.

MATERIALS AND METHODS

Animal Preparation and Recording Procedures

Experiments were performed on 10 adult cats, each weighing between 2 and 5 kg. Animal preparation followed an identical procedure to that described in Chapter 4 with the exception that an additional bilateral craniotomy was performed at HC coordinates +2.0 to -10.0mm anterior-posterior, and 0 to 6.0mm medial-lateral. The dura was then reflected to expose areas 17 and 18 in both hemispheres.

The visual stimuli consisted of moving light or dark bars presented, under computer control, in 20cm x 20cm raster via an endoscope. For each visually

stimulating region of the receptive field was probed by presenting a moving stimulus well outside the receptive field and gradually approaching its center.

Cooling Procedures Cooling of areas 17 and 18 was achieved with a Peltier thermoelectric device (28W; manufactured by Cambion, USA) attached to a silver contact foot. Temperature was measured by miniature thermocouples constructed from 25mm thick constantan and copper wires glued to tips of glass-coated tungsten microelectrodes and read from digital thermometers (accuracy of 0.1°C; Omega, USA; HH 72T). The silver contact foot, of dimension 12mm x 12mm, extended from HC: AP +2.0 to -10.0mm and ML 0 to 6.0mm into both hemispheres and covered a portion of the primary visual cortex known to represent centrally located receptive fields. Silicon grease provided thermal contact between the foot and the surface of the cortex. A thermocouple was inserted through a small opening in the foot on the ipsilateral side to record temperature in the ipsilateral primary visual cortex. A second thermocouple and the microelectrode were inserted into area 21a. Both thermocouples were positioned

1mm below the cortical surface. Fig. 6.1 illustrates an anaesthetised cat in the stereotaxic device and the relative positions of the cooling device, thermocouples and recording microelectrode.

Visual Stimulation and Recording Procedures

Extracellular single unit recordings were made with tungsten-in-glass microelectrodes with an impedance of 2-10 M Ω inserted into the cortex (Levick, 1972; Merrill and Ainsworth, 1972). Whilst most recordings were made from cells in area 21a, the response characteristics of a small sample of cells in area 17 were also recorded. This allowed comparisons to be made between the response features of cells in the two areas. The visual stimuli consisted of moving light or dark bars presented, under computer control, in 26cm x 26cm raster on an oscilloscope. For each visually responsive cell the activating region of the receptive field was plotted by presenting a moving stimulus well outside the receptive field and gradually approaching its centre. The borders between responsive and unresponsive regions were then drawn directly on a tangent screen.

Tests were made of the cells' orientation specificity, acuteness of orientation tuning, directional selectivity and, in the case of area 17 cells, velocity tuning. Procedure for performing these tests has been described in Chapter 4.

These tests were carried-out under conditions in which the temperature in the primary visual cortex was progressively lowered from its usual physiological level of 35.5°C. In a few experiments the temperature was reduced to the point at which the response of the cell in area 21a was blocked completely. However, in order to avoid permanent tissue damage through extreme cooling of the primary visual cortex,

FIGURE 6.1

Photograph of anaesthetised cat in stereotaxic device showing the relative positions of the thermoprobes, cooling device and recording electrode.

PELTIER COOLING DEVICE

THERMOPROBE (AREA 17)

SILVER CONTACT FOOT
(AREAS 17 & 18)



MICROELECTRODE
(AREA 21a)

THERMOPROBE
(AREA 21a)

temperature was reduced, in most cases, to the point where the response in area 21a was lowered but not abolished. The temperature in the primary visual cortex was then allowed to recover before further recording in area 21a was undertaken.

Sample

Results were obtained for a sample of 14 cells isolated in area 21a and 13 cells isolated in area 17. All cells had generally increased receptive fields; 85% of area 21a cells lay within 9° of the area centralia, as did all the cells of area 17. In addition, all cells were binocular and orientation selective.

Experimental Paradigm

Two experimental paradigms were used in this experiment, both of which employed cooling of the primary visual cortex (areas 17 and 18). In one paradigm cell responses were recorded in area 17 and in the other they were recorded in area 21a. The assessment of the cell's responsiveness relied heavily on the preparation of orientation tuning curves at different levels of cooling. Concentration was focused on orientation selectivity firstly because it was an outstanding response feature of the cells of both areas and secondly because the shape of the tuning curve would reflect the influence that cooling had on both the excitatory and inhibitory processes occurring in the primary visual cortex.

RESULTS

Sample

Results were obtained for a sample of 64 cells isolated in area 21a and 13 cells isolated in area 17. All cells had centrally located receptive fields; 88% of area 21a cells lay within 5° of the area centralis, as did all the cells of area 17. In addition, all cells were binocular and orientation selective.

Experimental Paradigm

Two experimental paradigms were used in this experiment, both of which employed cooling of the primary visual cortex (areas 17 and 18). In one paradigm cell responses were monitored in area 17 and in the other they were confined to area 21a. The assessment of the cell's responsiveness rested heavily on the preparation of orientation tuning curves at different levels of cooling. Concentration was focussed on orientation selectivity firstly because it was an outstanding response feature of the cells of both areas and secondly because the shape of the tuning curve could reflect the influence that cooling has on both the excitatory and inhibitory processes occurring in the primary visual cortex.

Temperatures and Temperature Gradients

The temperature of area 17 measured at the beginning of the experiment varied between 34°C and 36°C. By comparison, the cat's body temperature, measured with a thermoprobe inserted deep under the scapula, was around 37.5°C. Later when a craniotomy over the primary visual cortex was enlarged and the cooling probe inserted, the cortex temperature was maintained at 35.5°C by using a small heating current to compensate for any heat loss, that otherwise could reduce the tissue temperature. Tests indicated that without such compensation the temperature of the primary visual cortex would have fallen to as low as 29°C.

The size and the position of the cooling foot was designed to fit maps of the cortex (Bilge et al., 1967; Tusa et al., 1979) in an attempt to achieve direct cooling over the region representing the central part of the visual field (including the area centralis and out to 5 deg. of eccentricity). The surface of the cortex directly beneath the cooling probe had a uniform temperature. Experiments were performed on two cats, however, to measure the temperature of the cortex at various depths. This revealed that the temperature of the tissue increased with depth. For example, when the temperature of cortical tissue at a depth of 1mm had been lowered to 10°C, the temperature of tissue 1mm deeper was 4°C to 5°C higher. However, because the cortex is relatively thin and insertion of the thermocouple caused the tissue to "dimple", it was not possible to construct an accurate table of temperature gradients.

In recording sessions the temperature of areas 17 and 18 was lowered gradually and, to avoid damage, cooling was usually terminated when the cell activity dropped below 20% - 30% of its original value. In 15 cases however, the temperature of areas

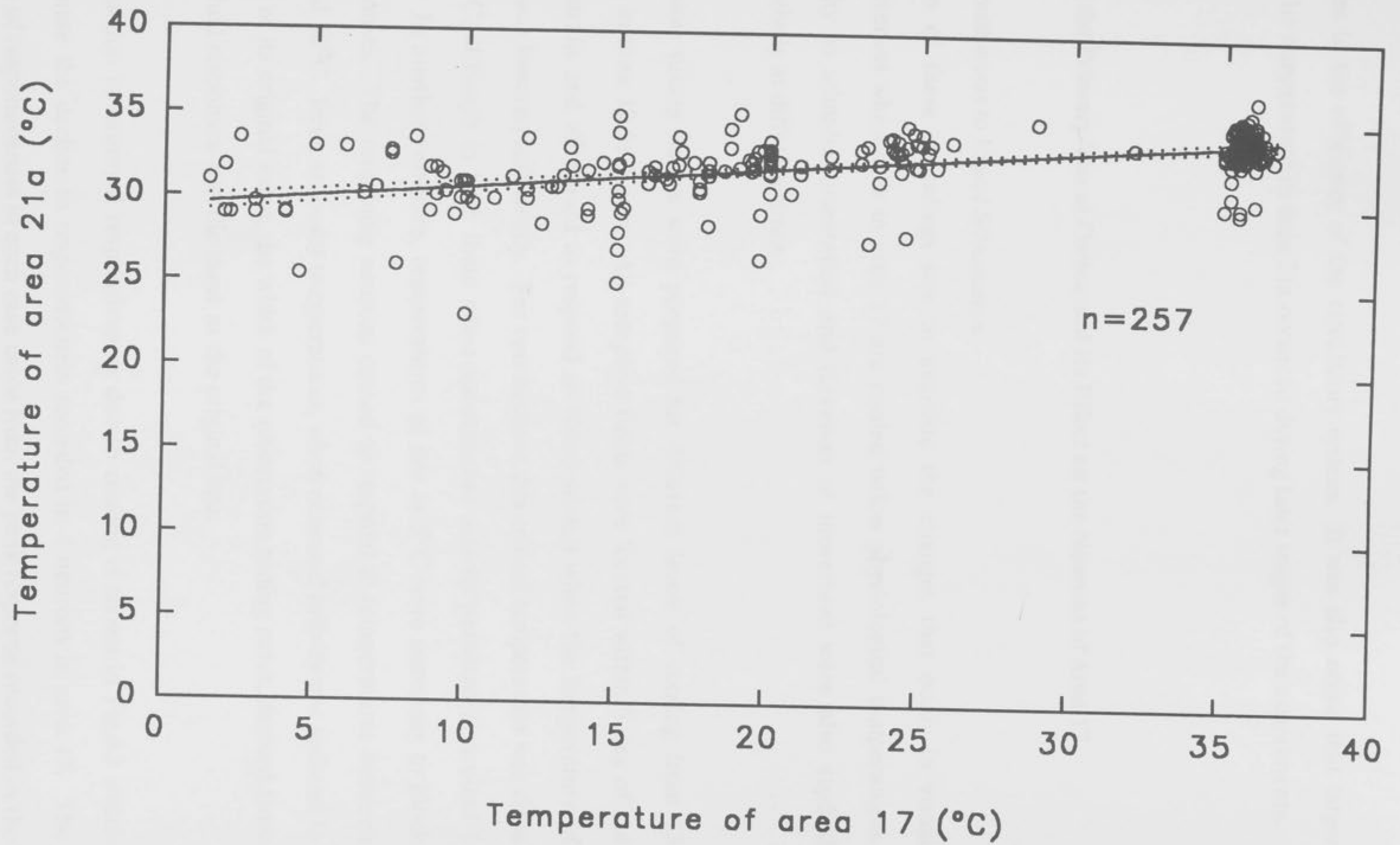
17 and 18 was lowered to 2°C - 7°C , a temperature where earlier findings suggested that all neuronal activity would be blocked.

The relatively small craniotomy used to expose area 21a and the insulation provided both by the metal well glued to skull and the agar coating on the cortex, resulted in relatively little heat loss from this area. A critical issue, however, is whether the spread of cooling from the primary visual cortex influenced the level of response of the cells in area 21a. To determine whether there was any spread of cooling from the primary visual cortex to area 21a, the temperature of area 21a was monitored constantly throughout the experiments to detect . Figure 6.2 shows the temperatures of area 21a as a function of the temperature of the primary visual cortex prepared from 257 concurrent measurements in the two areas. The linear regression coefficient (r) calculated for the 257 temperature pairs was 0.12. For the most extreme cooling of areas 17 and 18 (a reduction of around 30°C to a level of 3°C), the mean temperature fall in area 21a was 4°C . The greatest temperature fall recorded in area 21a during cooling of the primary visual cortex was 7.3°C but this applied only to a single cell. In only 13 neurons (20% of the sample) did the fall in area 21a exceed 3.5°C and in eight of these the decline did not exceed 5°C . At the other end of the scale, the temperature decline did not exceed 2°C in 11 neurons (17%). Furthermore, recording experiments confined to area 17 neurons revealed that temperature reductions in excess of 5°C were required to generate a fall in neuronal activity. Using these results as a guide, it is assumed that a similar reduction in area 21a temperature would have been required to cause a decline in neuronal responsiveness in that area.

The temperature of area 21a as a function of temperatures in the primary visual cortex varied slightly between animals. This was probably attributable to individual

FIGURE 6.2

The direct effect of cooling area 17 on the temperature measured simultaneously in area 21a. The near horizontal regression line shows a low level of temperature dependence (coeff. of correl., $r = 0.12$; $n = 257$ temp. pairs). Typically, when area 17 was cooled to 2°C the temperature in area 21a would fall only to around 30°C but the starting temperature of the agar-protected cortex was between 35°C and 31°C . In 80% of area 21a neurons the largest temperature change with difference due to cooling was smaller than 3.5°C .



variations in the efficiency of the circulatory system. It was also noted that larger falls in the temperature in area 21a occurred during later stages of the experiments.

Cooling the Primary Visual Cortex and its Effect on the Neurons of Area 17.

1. Responsiveness to Visual Stimulation.

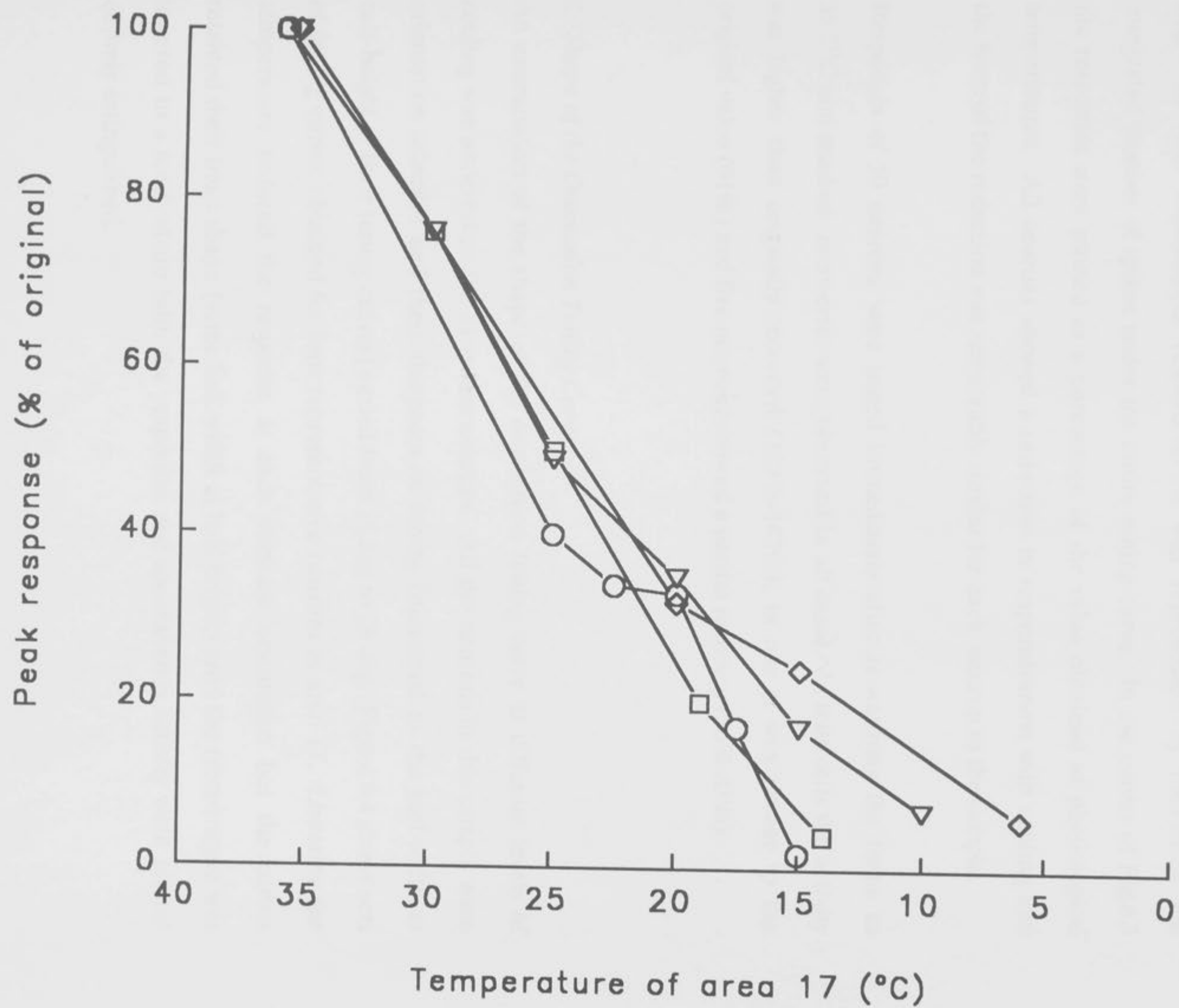
The aim of these recordings was to evaluate the changes that occur in visual responsiveness when cells in area 17 are cooled below physiological temperatures. Selectivity to stimulus orientation and direction of movement were also studied quantitatively at different times.

Orientation tuning curves were prepared for different levels of cooling from 13 neurons in area 17 in 3 cats. All receptive fields were located within 5 deg of the area centralis and all ceased to respond to visual stimuli when the temperature of area 17 was lowered sufficiently. For two neurons this critical temperature was close to 18.0°C, although in both these cases spontaneous activity persisted at around 1 spike/s. In another two units, temperatures as low as 5°C were necessary to block the responses. The remaining neurons ceased to respond at temperatures between 10°C and 16°C. Even at lowest temperatures, when neuronal activity was reduced to only 5% of its original value, the width of the orientation tuning curve, derived from the residual responses, was the same as the original one.

The reduction in neuronal responsiveness due to cooling is shown in Fig.6.3 which demonstrate the decline in responsiveness recorded in 4 neurons in area 17. The measure of responsiveness in each case came from the peak response recorded in the orientation tuning curve for each temperature of the primary visual cortex. Each

FIGURE 6.3

The responsiveness (peak mean response in the orientation tuning curve) of four directly cooled neurons in area 17 as a function of temperature. The consistent nature of the decline in responsiveness in each of the four cells was a common feature of direct cooling in area 17.



tuning curve was derived from average response histograms prepared from 5 presentations of a bar stimulus set at 10 different orientations each separated by 10 deg. In later experiments responsiveness was represented by measuring the integrated number of spikes under the entire tuning curve. In the curves of Fig.6.3 the responses were plotted as a percentage of the value obtained at physiological temperatures. All neurons showed a reduction in responsiveness with cooling and the form of the reduction was remarkably similar for each neuron in the sample.

Responses of 10 neurons were tested immediately after re-warming the tissue to 35.5°C and marked recoveries were observed in all cases. In four cells the activity was higher than originally recorded (103%-142%); in one it was similar to the original value (91%) and five neurons showed a partial recovery (50%-83%).

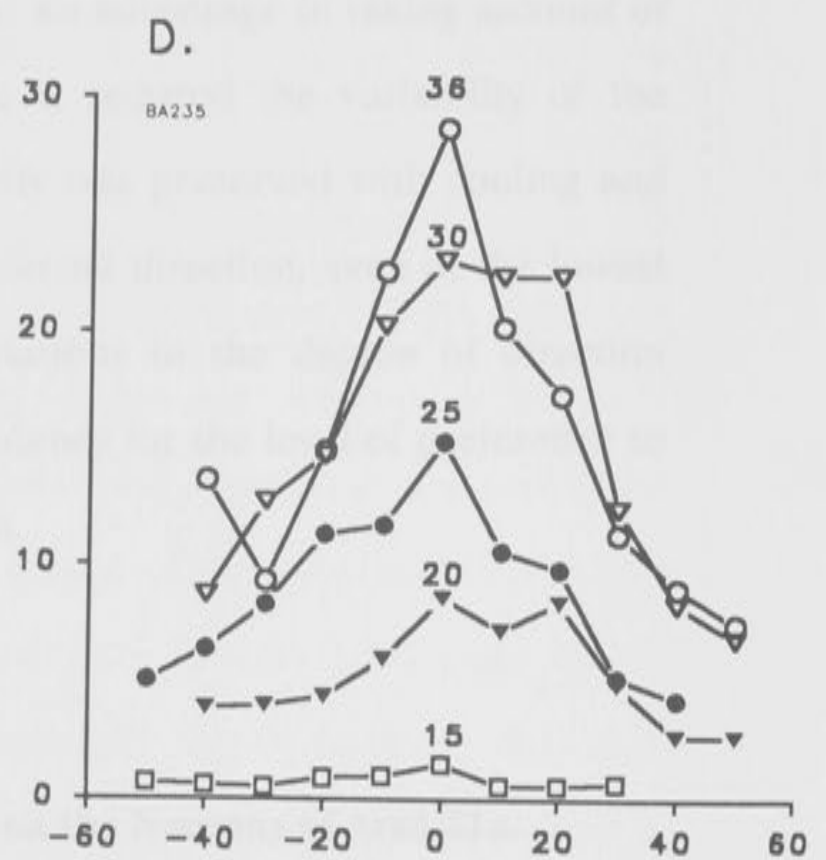
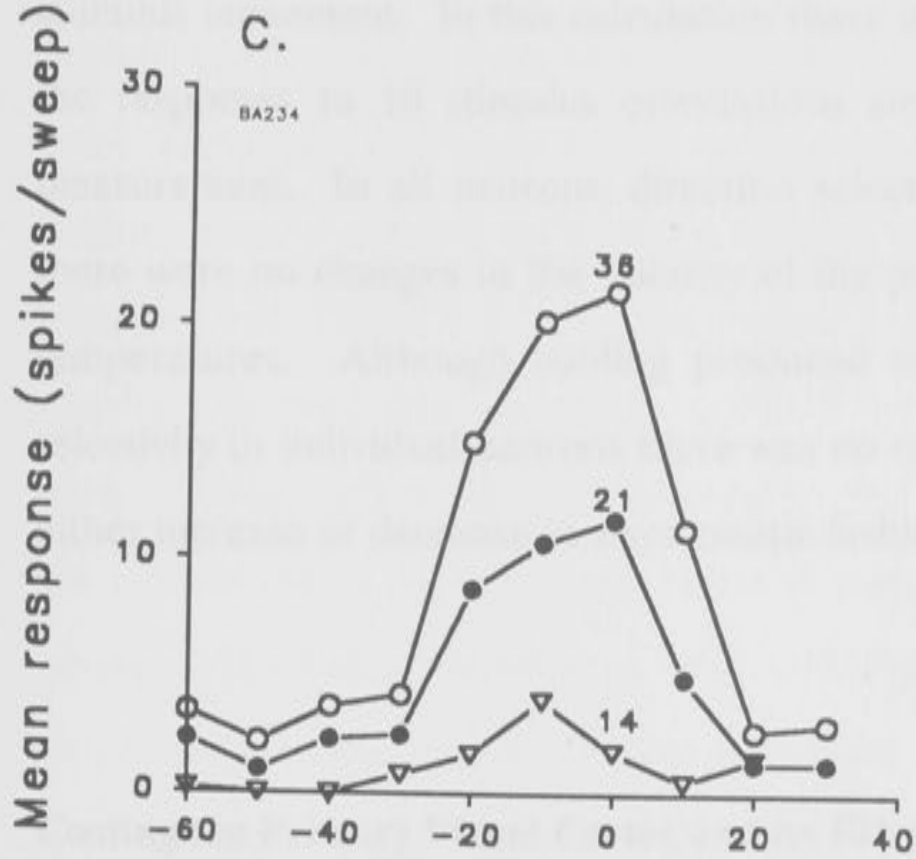
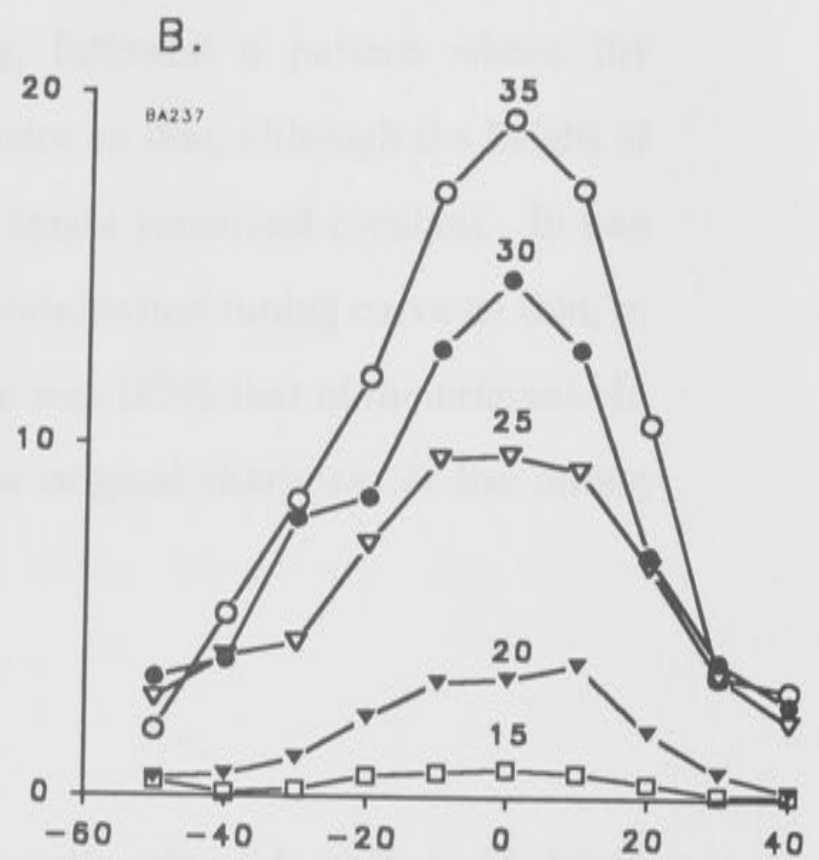
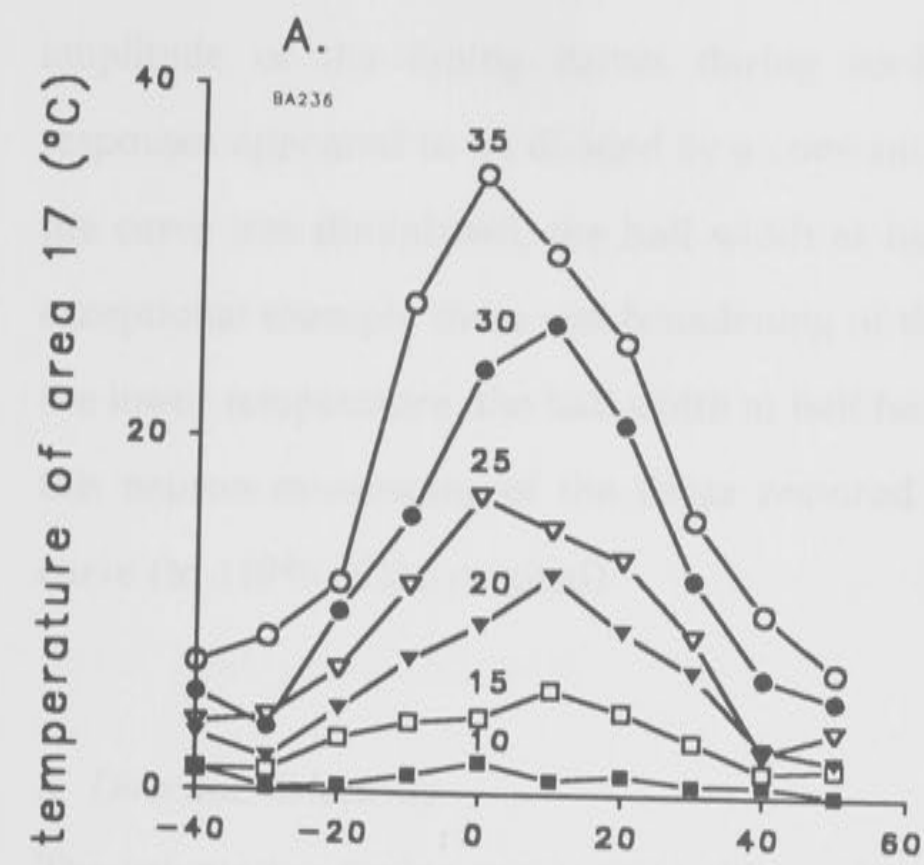
2. Shape of the Orientation Tuning Curve

An examination of the shape of the orientation tuning curve at different levels of cooling was undertaken for 11 striate neurons. All the neurons in this sample were orientation selective and their sharpness of tuning (measured as the half-width at half-height of their tuning curves) varied from 10 deg to 28 deg. Figure 6.4 shows sets of tuning curves obtained for four representative neurons in area 17. Lowering the temperature reduced the response at each stimulus orientation but the curves retained their basic shape (same half width at half height) until the temperature was lowered to a level where both the responses and spontaneous activity were almost entirely extinguished.

The retention of the width of the orientation tuning curve during cooling was a common feature in striate neurons and it held even when the individual responses

FIGURE 6.4

Orientation tuning curves of four area 17 neurons plotted at different cortical temperatures. Note, at each cooling step, the divisive nature of the change that reduced the height of the curve without altering its width. Note also that orientation selectivity persisted at very low levels of responsiveness (i.e. at temperatures around 15°C).



Orientation of stimulus (deg)

Mean response (spikes/sweep) at given temperature of area 17 (°C)

were reduced to as low as 5% of the initial value. In 10 neurons, the reduction in amplitude of the tuning curves during cooling, followed a pattern where the responses appeared to be divided by a constant factor so that, although the height of the curve was diminished, the half width at half height remained constant. In one exceptional example there was broadening of the orientation tuning curve so that, at the lower temperature, the half width at half height was 187% that of the original. In this neuron re-warming of the tissue restored the original sharpness of the tuning curve (to 119% of the original).

3. *Direction Selectivity.*

The orientation tuning curves were also analysed for direction selectivity in 11 striate neurons. The degree of direction selectivity was measured as the ratio of the area under the curve for each of the two directions (preferred and non-preferred) of stimulus movement. In this calculation there was an advantage in taking account of the responses to 10 stimulus orientations since it reduced the variability of the measurement. In all neurons, direction selectivity was preserved with cooling and there were no changes in the polarity of the preferred direction, even at the lowest temperatures. Although cooling produced variations in the degree of direction selectivity in individual neurons there was no tendency for the level of preference to either increase or decrease in a systematic fashion.

Cooling the Primary Visual Cortex and its Effect on the Neurons of Area 21a.

1. *Responsiveness to Visual Stimulation.*

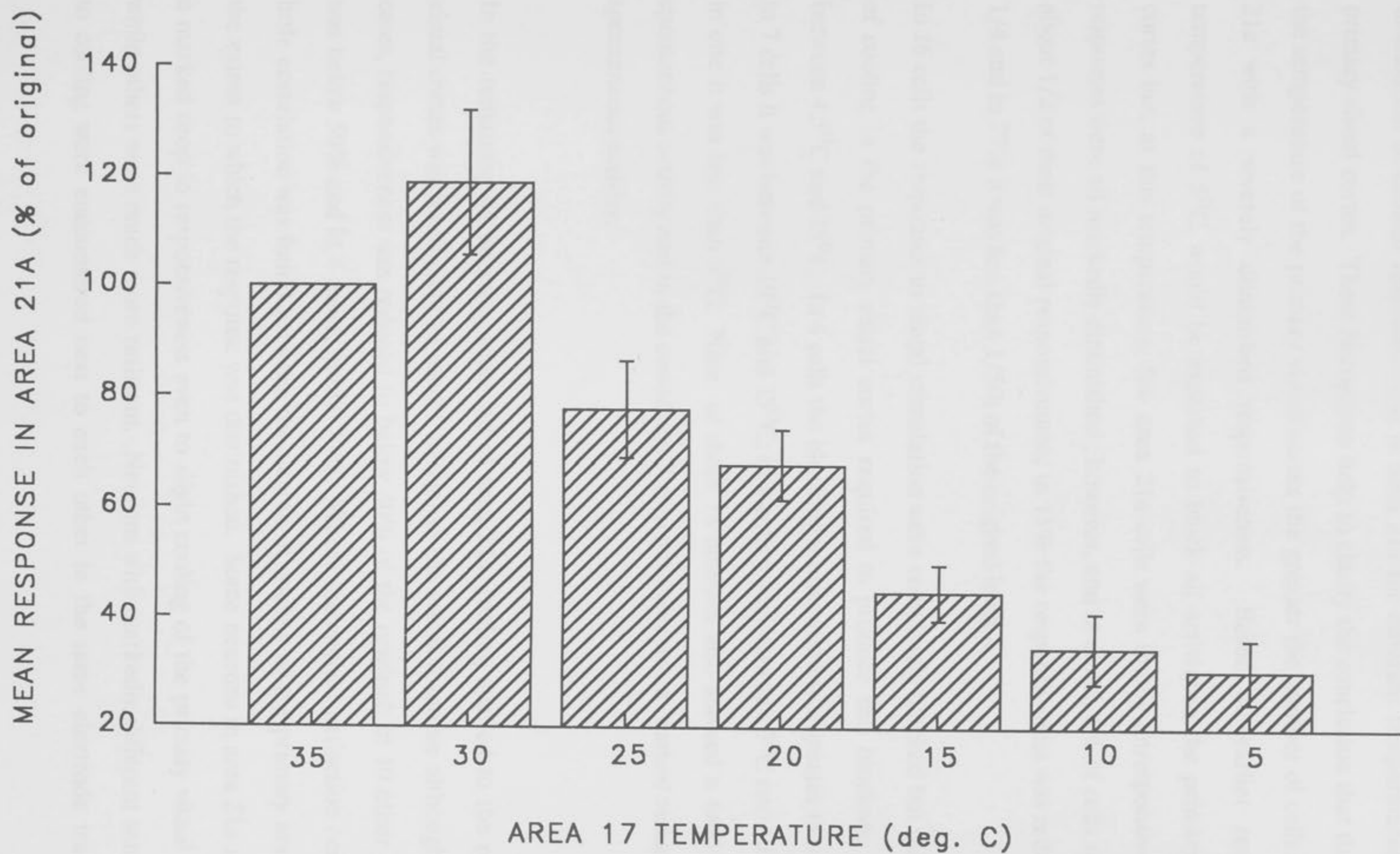
Response properties were recorded for 64 neurons in area 21a while the temperature in the primary visual cortex was successively lowered to different levels. Neuronal

receptive fields varied in size from 1 deg² to 26 deg². The receptive field centres of 52 neurons were located within 5 deg of the area centralis. For the remaining 12 neurons the eccentricity of their receptive field centres varied from 5.9 deg to 7 deg. The receptive fields of 34 neurons were entirely within the central 5 deg of the visual field while those of 24 neurons were partially within the 5 deg limit but did not extend beyond the 8 deg circle. Finally, 6 other neurons had receptive fields located entirely outside the central 5 deg and extending up to 9.6 deg. The neurons, with the most peripheral receptive fields, were included in the analysis because they showed typical effects associated with cooling of the primary cortex.

In all of the 64 analyzed cells in area 21a the visual response was markedly reduced when the primary cortex was cooled. In 14 cells, however, the initial step in cooling the primary visual cortex, to between 20°C and 30°C, resulted in an increase in responsiveness. The largest observed enhancement within this range of cooling was 209% although the mean for all cells was only 13.8%. When subject to further cooling, however, these cells always showed reduction of responsiveness below the original level. Figure 6.5 shows the levels of responsiveness attained by the sample of neurons in area 21a when the primary visual cortex was cooled to different temperatures. Here, the responsiveness was measured from the area under the orientation tuning curve and expressed as a percentage of the uncooled finding (see above). The mean response at each temperature level in area 17 is given by the height of the bars, and error bars represent variability in the response data. Of special note is the increase in mean responsiveness recorded at the first decline in temperature to 30°C. Neuronal responsiveness resulting from re-warming the cortex is not included. In the following figures (Figs.6.6, 6.7 & 6.8) extracts from these data are subject to further analysis.

FIGURE 6.5

The responsiveness (mean response: obtained by integrating the area under orientation tuning curve and expressed as % of original), of 64 neurons in area 21a, plotted as a function of temperature of area 17. Response data relates to experiments in which the temperature of the primary visual cortex was progressively reduced from 35°C and the standard error bars represent data variability at temperature levels below 35°C. Note that the mean responsiveness of area 21a cells increased with the initial fall in area 17 temperature and declined thereafter.



In an alternative presentation of the same data in Fig.6.6, bar histograms show the distribution of cellular responsiveness in area 21a for different temperatures of the primary visual cortex. These histograms help to clarify the conclusion that the lower the temperature of the primary visual cortex the greater the number of cells in area 21a with a severely diminished responsiveness. Based on earlier results, a temperature of 5°C , would be expected to block all activity in the primary visual cortex but, at this temperature, few area 21a cells were totally unresponsive. The responses were all markedly diminished, however, and in detail, 6% of cells retained about 1/2 of their original responsiveness; in 15% the responsiveness was reduced to 1/4 and in 77% it was less than 1/5th of the original level.

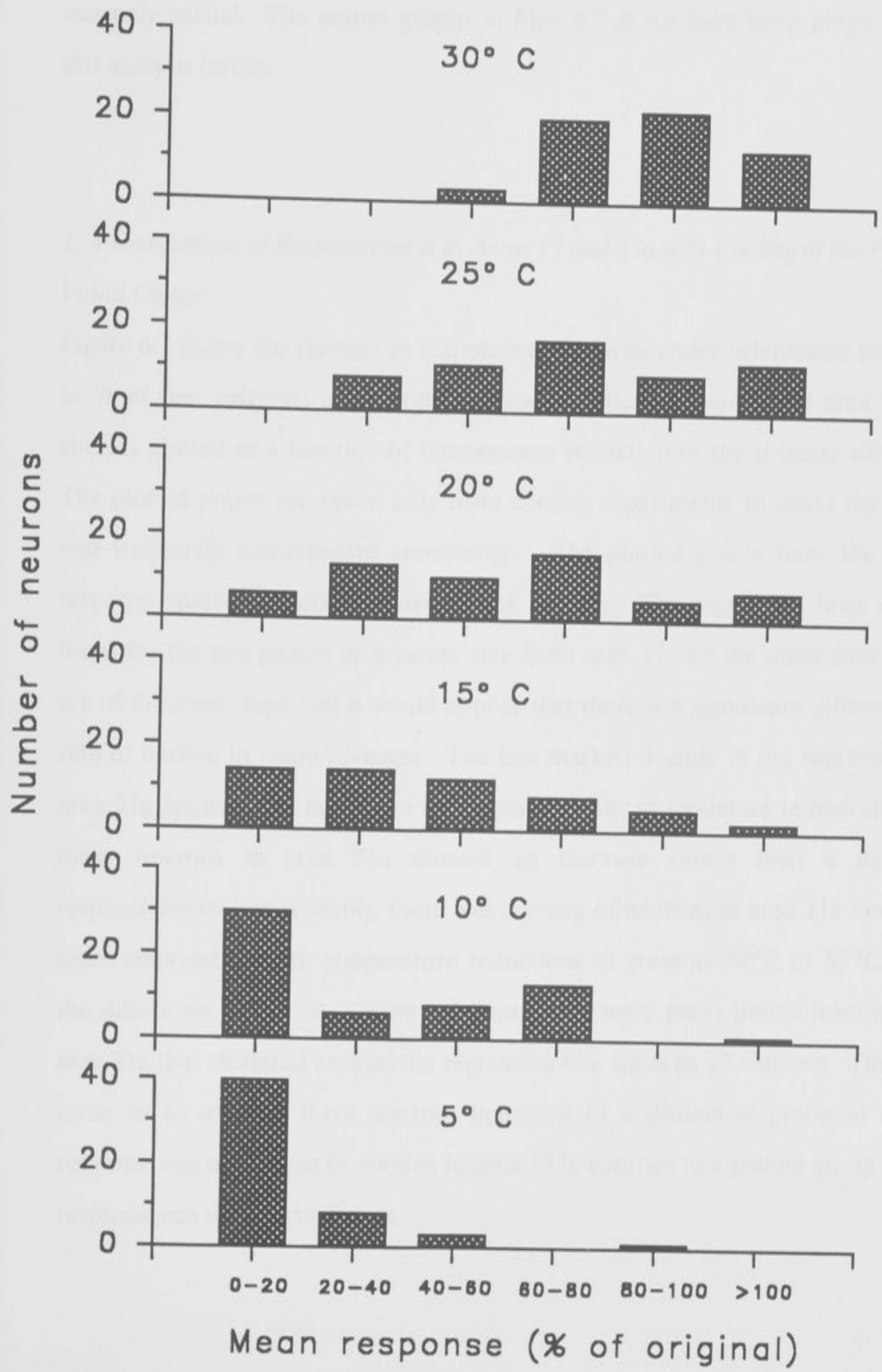
In 16 cells the responses to visual stimulation were completely blocked but the levels of cooling in the primary visual cortex required to produce this blockade ranged between 4.5°C and 25°C . In 4 cells the blocking temperature was greater than 20°C ; in 7 cells it was between 10°C and 15°C ; in 4 cells it was between 5°C and 10°C and in one it was less than 5°C . Nine of these 16 neurons also showed a total loss of spontaneous activity and in the remaining 7 neurons there was a marked reduction in spontaneous activity.

In the remaining 48 neurons, recorded in area 21a, cooling applied to the primary visual cortex was insufficient to totally extinguish the visual response although, in 34 cases, responsiveness was reduced to below 30% of the original; in 10 other cells it was below 50% and in 4 more, below 70%. Where incomplete extinction occurred, little correlation was found between the degree of cooling of the primary areas and the extent to which the response was diminished. Some neurons in area 21a showed a marked drop in responsiveness even to slight cooling of the primary visual cortex while others were much more resistant. Neurons with markedly different sensitivity to cooling were encountered next to each other in the same electrode track. It

FIGURE 6.6

Distribution histograms, derived from area 21a neurons, showing how the level of responsiveness declines in the sample (the same sample and measure of responsiveness as Fig. 6.4) with the cooling of the primary visual cortex.

selected group of cells were present in area 21; the first could be blocked totally by cooling the primary visual cortex, and the other were not working at all. The scatter graphs in Fig. 5, 6 & 7 have been prepared to take the analysis further.



seemed possible that two groups of cells were present in area 21a: one that could be blocked totally by cooling the primary visual cortex, and the other where the blocking was only partial. The scatter graphs in Figs. 6.7 & 6.8 have been prepared to take this analysis further.

2. A Comparison of Responsiveness in Areas 17 and 21a with Cooling of the Primary Visual Cortex

Figure 6.7 shows the changes in responsiveness (area under orientation tuning curve as % of the original) of neurons in area 17 (filled triangles) and area 21a (open circles) plotted as a function of temperature reduction of the primary visual cortex. The plotted points are taken only from cooling experiments to avoid the hysteresis that frequently accompanied rewarming. The plotted points show the scatter of response values for different degrees of cooling. The regression lines (unbroken lines) for the two groups of neurons, one from area 17 and the other from area 21a, are of different slope and it would appear that there is a significant difference in the rate of decline in responsiveness. The less marked decline in the regression line in area 21a appeared to be due to two factors: firstly, at moderate temperature drops, many neurons in area 21a showed an increase rather than a decrease in responsiveness; and secondly, there was a group of neurons in area 21a that retained some response even to temperature reductions as great as 30°C or 35°C. Despite the difference in the two regression lines, there were many points from neurons in area 21a that clustered around the regression line for area 17 neurons. The question arose as to whether these neurons belonged to a distinctive group in which the response was totally lost by cooling in area 17 in contrast to a second group where the response was only partially lost.

FIGURE.6.7

Superimposed graphs showing the responsiveness of area 17 neurons (filled triangles) and area 21a neurons (open circles) as a function of the temperature of the primary visual cortex. Data points derived only from cooling experiments. First order regression lines shown as solid lines and 95% confidence intervals as dashed lines. The 95% confidence interval is the numeric range within which, for a given temperature, the mean of the data points will lie with a probability of 0.95.

Little overlap of the confidence lines suggests that the difference between the positions of the regression lines is statistically significant. The two correlation coefficients represented by the regression lines are:

$$\text{area 17 temp./area 17 activity} = -0.7$$

$$\text{area 17 temp./area 21a activity} = -0.3$$

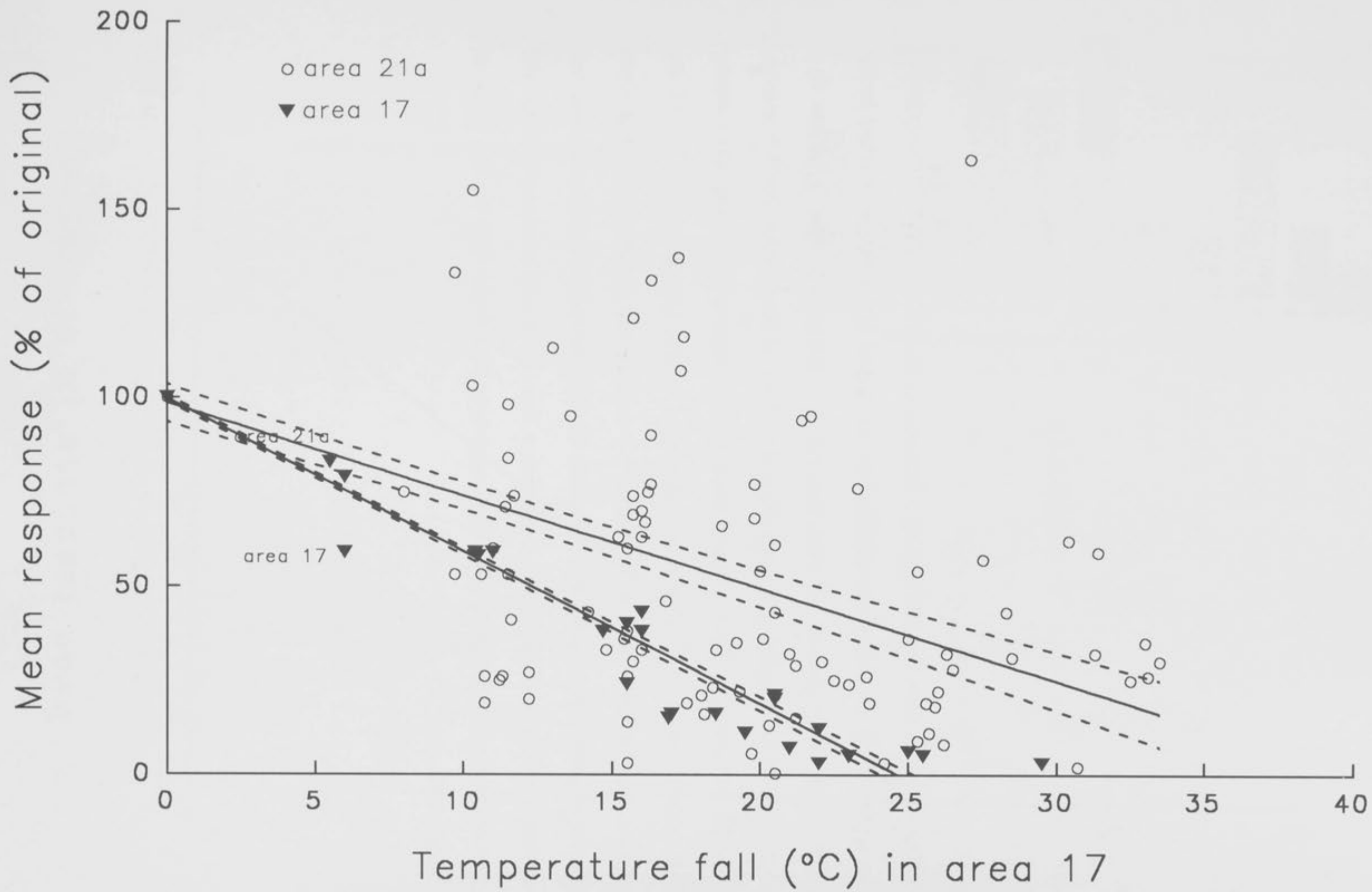


FIGURE 6.8

Responsiveness of area 21a neurons as a function of the temperature of the primary visual cortex. Only the data corresponding to the lowest temperature reached in the primary cortex were plotted for each neuron. For comparison the solid line is a reproduction of the regression line showing the effect of cooling on area 17 neurons in Fig. 6.6. All area 21a neurons were made less responsive by cooling the primary visual cortex, some more so than typical area 17 cells, (points to the left of the regression line) while others were more resistant (points to the right). The inset shows the distribution of units with different displacements (perpendicular distance in arbitrary units) from the area 17 regression line. Note one peak centred on the regression line and another to the right suggesting that there may be two types of neurons in area 21a.

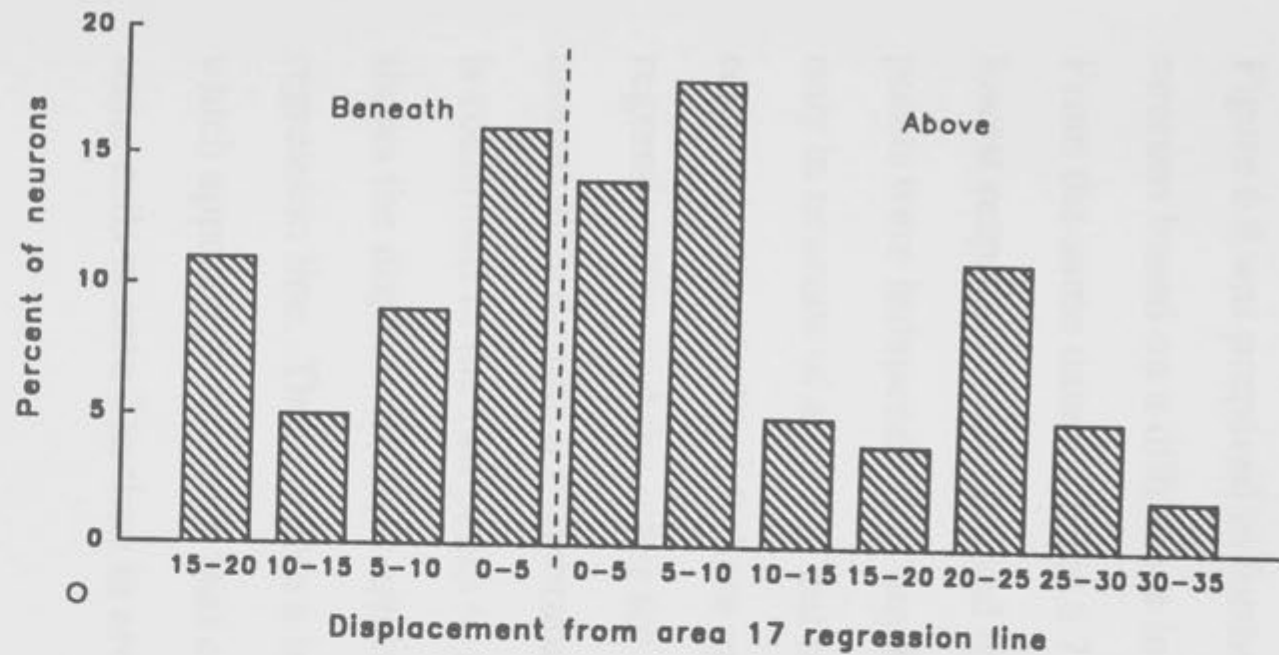
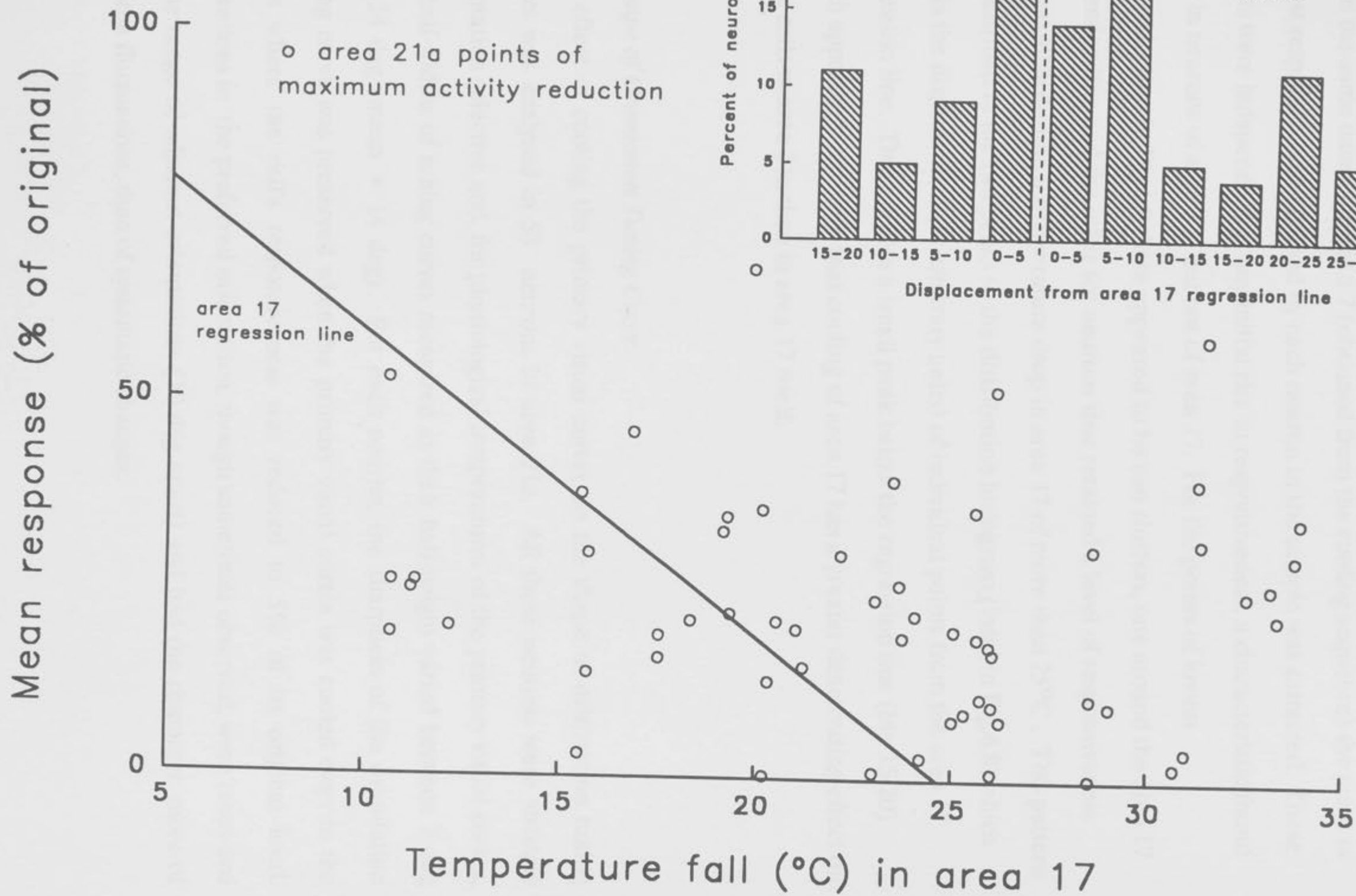


Figure 6.8 was prepared to further explore the possibility of two groups of area 21a neurons based on a difference in their dependence on the primary visual cortex. From the same data as in Fig.6.7 (obtained from the cooling sequence) the point of lowest responsiveness attained by each neuron in the sample was extracted. These points were independent of any initial rise in responsiveness, a characteristic found only in neurons of area 21a but not of area 17. For the points of lowest responsiveness in Fig.6.8 there appeared to be two clusters, one around the area 17 regression line and the other for neurons that retained a level of responsiveness around 40% despite a temperature drop in area 17 of more than 25°C. This pattern is confirmed in the two peaks in the distribution histogram (inset in Fig.6.8) which shows the displacement (in arbitrary units) of individual points from the area 17 regression line. There is also a small peak below the regression line (bin 15-20) which apparently indicates that cooling of area 17 has a greater deactivating effect on some cells in area 21a than in area 17 itself.

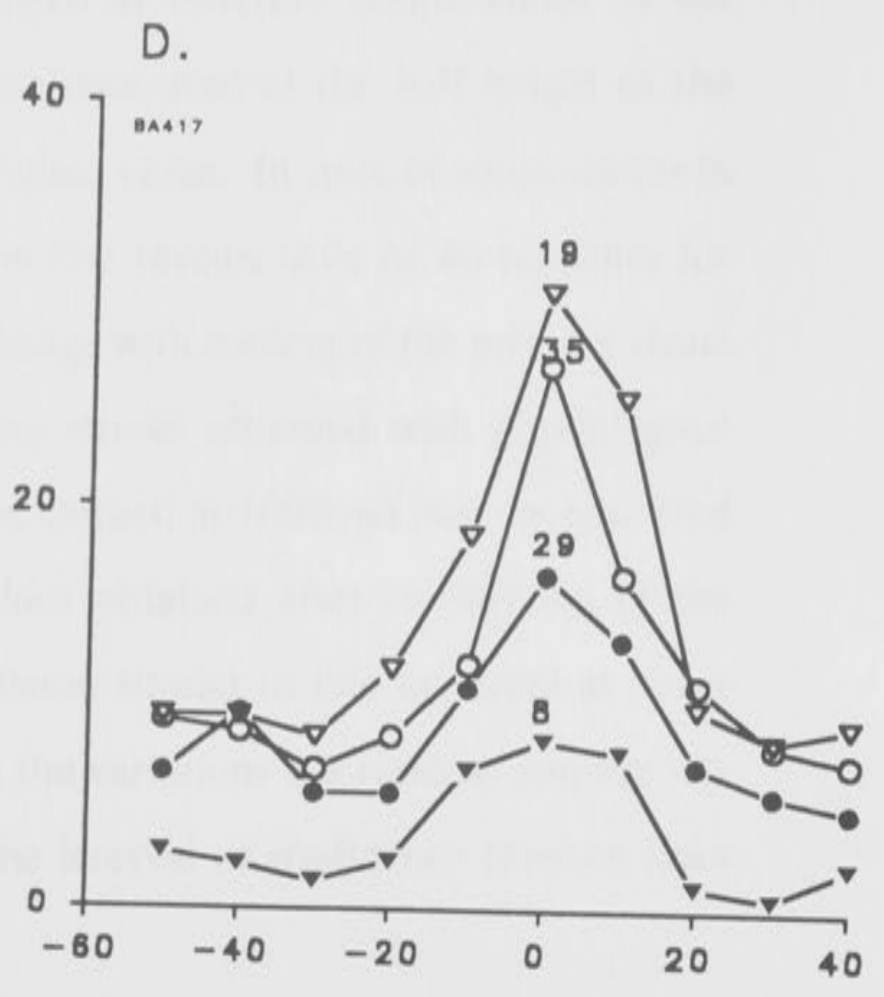
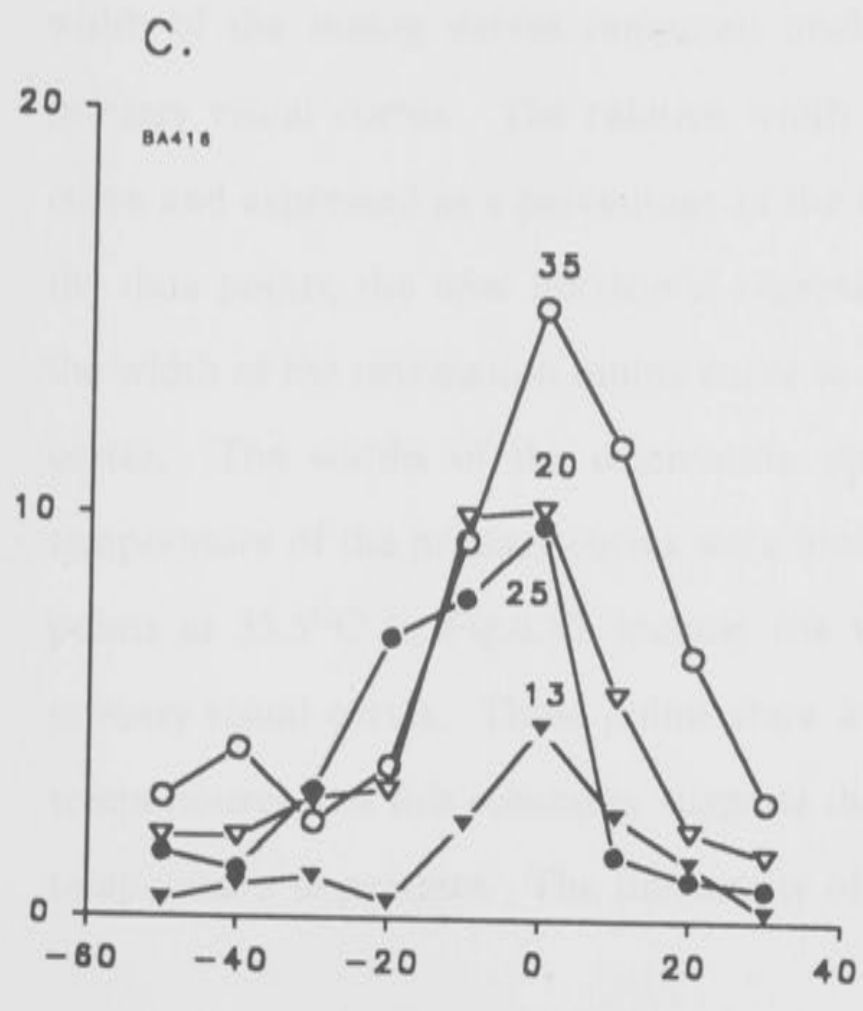
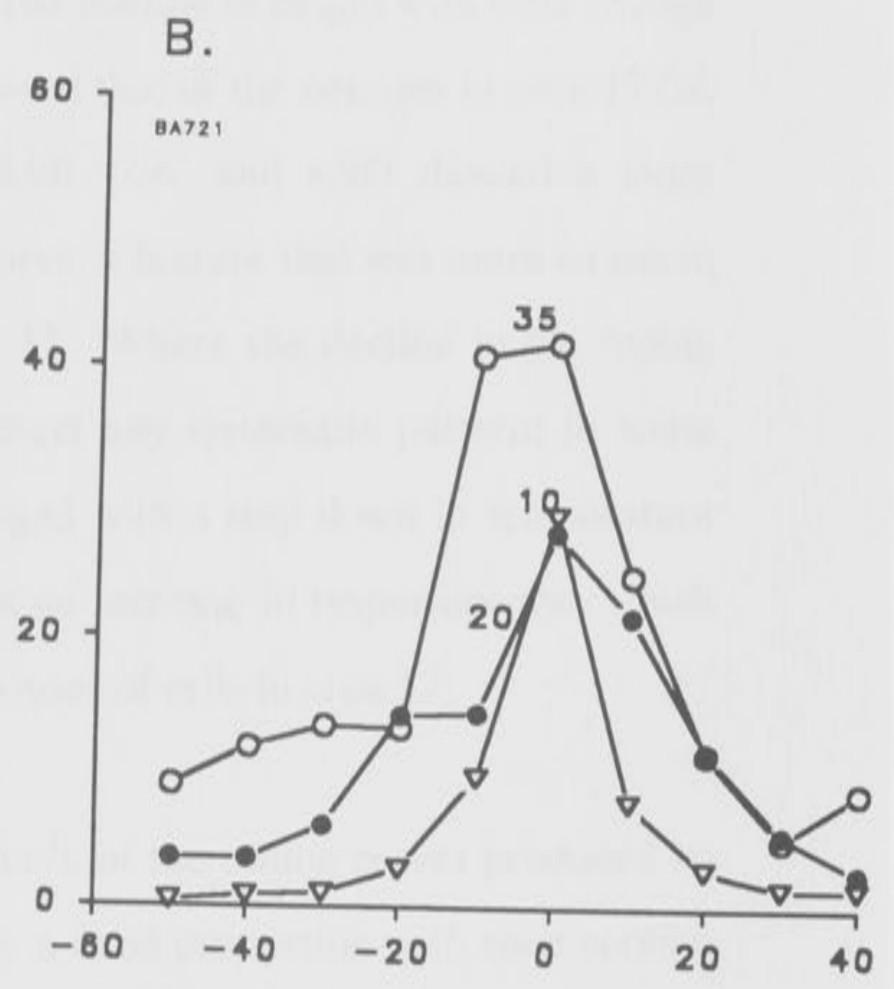
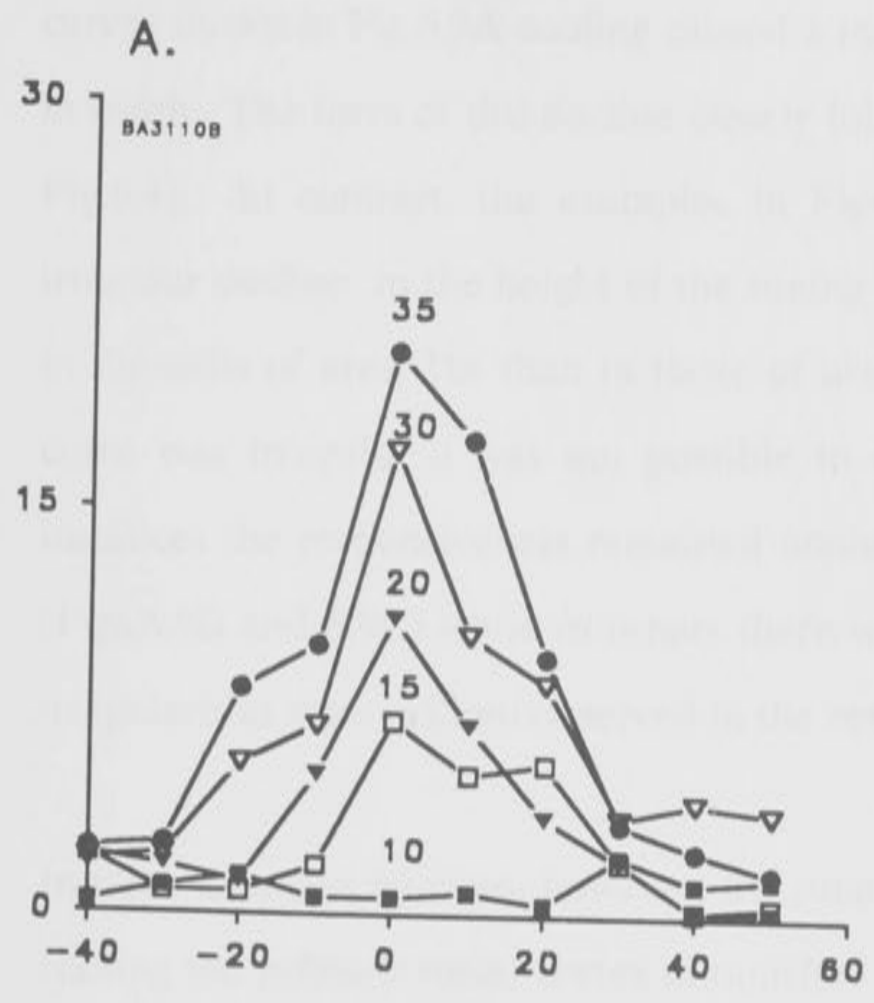
3. Shape of Orientation Tuning Curve.

The effect of cooling the primary visual cortex on the shape of orientation tuning curves was analyzed in 58 neurons in area 21a. All these neurons were strongly orientation selective and, for physiological temperatures of the primary visual cortex, the half-widths of tuning curves measured at their half-height varied between 7 deg and 24 deg (mean = 14 deg). For each neuron, the sharpness of the orientation tuning curve was preserved when the primary visual cortex was cooled even to the point where the cell's responsiveness was reduced to 5% of its original level. Alterations in the preferred orientation, though sometimes observed, were restricted to the range of adjacent orientations (10 deg apart) and had the character, more of random fluctuations, than of systematic changes.

FIGURE.6.9

Orientation tuning curves of four area 21a neurons plotted at different temperatures of the primary visual cortex. The orientation tuning curves of these, and all other neurons tested in area 21a, declined in the divisive manner reported earlier in Fig. 6.4 for directly-cooled neurons in area 17.

Mean response (spikes/sweep) at given temperature of area 17 (°C)



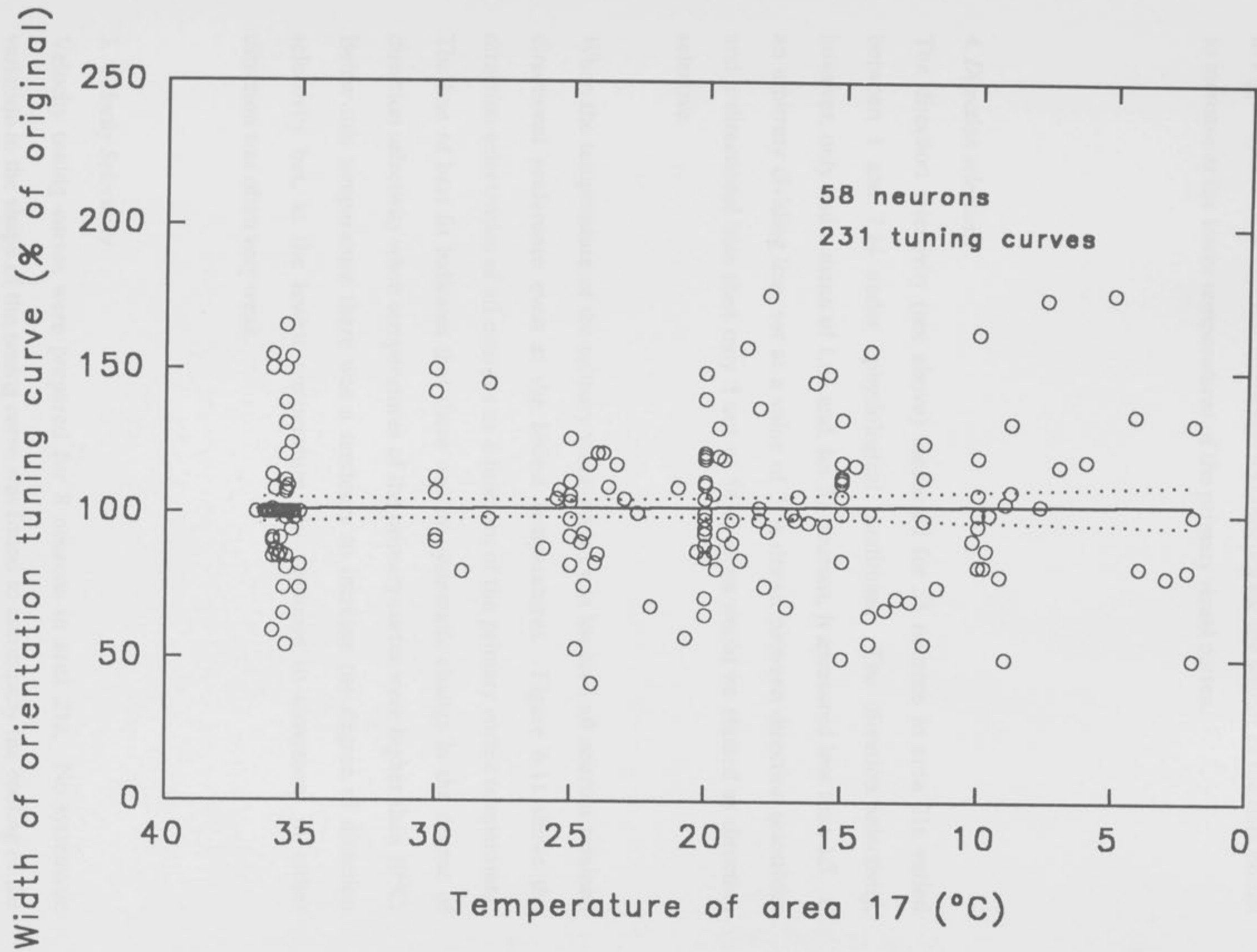
Orientation of stimulus (deg)

Figure 6.9 shows sets of tuning curves for four area 21a neurons obtained for different levels of cooling of the primary visual cortex. For the family of tuning curves shown in Fig.6.9A cooling caused a regular decline in height with little change in width. The form of this decline closely followed that of the neurons in area 17 (cf. Fig.6.4). In contrast, the examples in Figs.6.9B, 6.9C and 6.9D showed a more irregular decline in the height of the tuning curve, a feature that was more common in the cells of area 21a than in those of area 17. Where the decline in the tuning curve was irregular it was not possible to detect any systematic pattern; in some instances the responsiveness remained unchanged with a step down in temperature (Figs.6.9B and 6.9C) while in others there was an increase in responsiveness. Such irregularities were seldom observed in the responses of cells in area 17.

In most area 21a neurons, however, the amplitude of the tuning curves produced by cooling the primary visual cortex diminished by a fixed proportion with each cooling step. To discover how frequently this process of "diminishing through division" occurred in the sample of area 21a cells Fig.6.10 was prepared to see if the relative width of the tuning curves remained unchanged at different temperatures of the primary visual cortex. The relative width was measured at the half height of the curve and expressed as a percentage of the original value. In spite of some scatter in the data points, the near horizontal regression line reveals little or no tendency for the width of the orientation tuning curve to change with cooling of the primary visual cortex. The widths of the orientation tuning curves obtained with physiological temperature of the primary cortex were always defined as 100% so that the scattered points at 35.5°C in Fig.6.10, include the values obtained after re-warming of the primary visual cortex. These points show a scatter similar to that apparent at lower temperatures and this constancy suggests that the variations are random and are not temperature dependent. The uniformity of the interval of confidence (broken lines

FIGURE 6.10

The effect that the cooling of the primary visual cortex has on the sharpness of the orientation tuning curve (half width at half height) of cells in area 21a. In spite of considerable scatter the near horizontal regression line (solid) and 95% confidence intervals (dotted lines) reveal a tendency to preserve the original sharpness of tuning. Scattered points corresponding to a temperature of 35.5°C were obtained after re-warming the primary visual cortex; the scatter of these points provides a control since it was comparable to that observed during the cooling experiments.



in Fig.6.10) also indicates that there was no tendency for the scatter of the data points to increase at the lower temperatures of the primary visual cortex.

4. Direction selectivity

The direction selectivity (see above) measured for 58 neurons in area 21a varied between 1 and 7.14 under physiological conditions. The direction selectivity, however, only had a mean of 1.66 and, for 37 neurons, it measured less than 1.5. If an arbitrary dividing line, set at a value of 3, is drawn between direction selectivity and a directional bias then only 5 out of 58 neurons would be classed as direction selective.

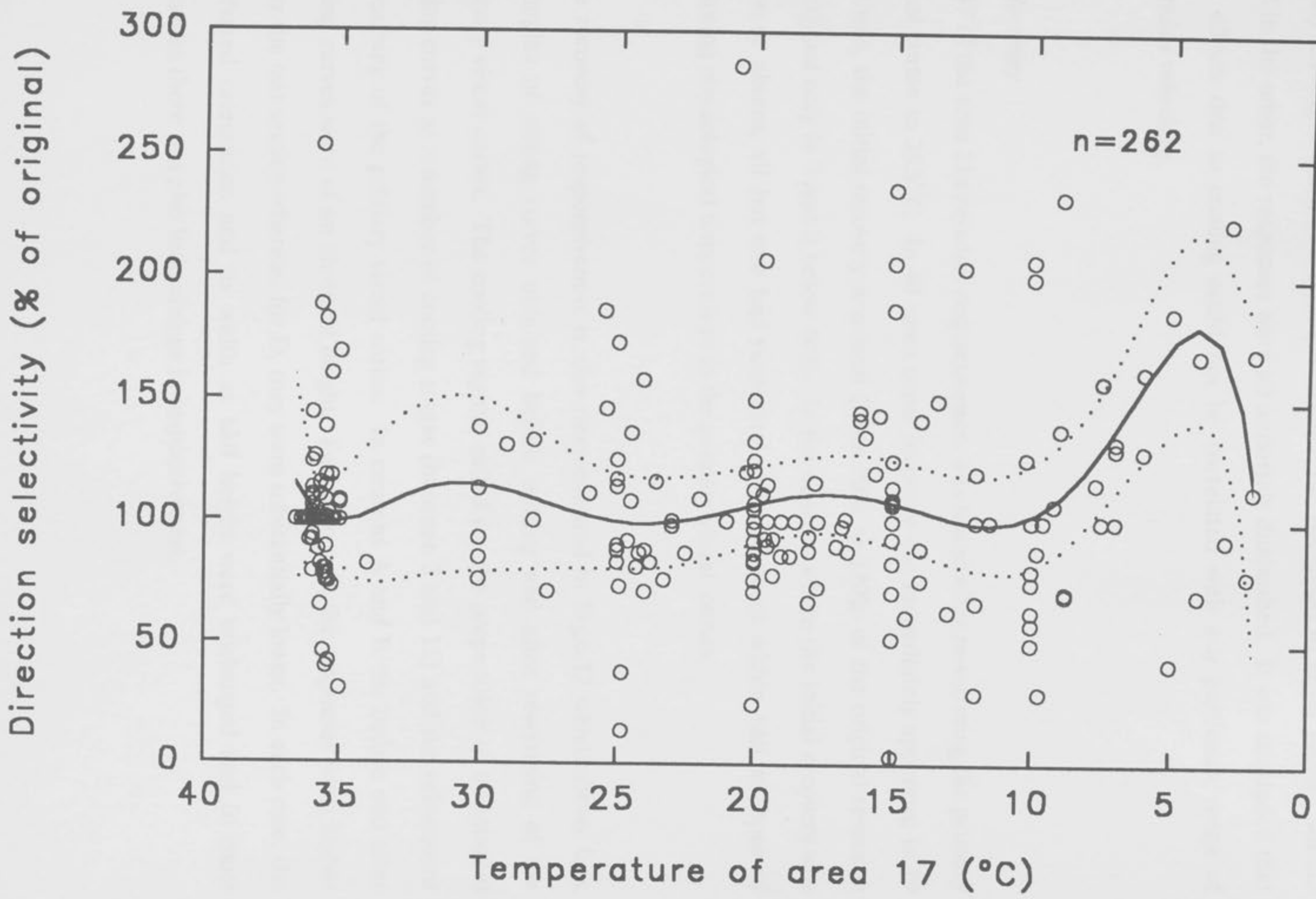
When the temperature of the primary visual cortex was lowered all neurons retained directional preference even at the lowest temperatures. Figure 6.11 shows the direction selectivities of all neurons as a function of the primary cortex temperature. The line of best fit indicates that there was no systematic change in the degree of direction selectivity when temperatures of the primary cortex were higher than 10°C. Below this temperature there was a tendency to increase the degree of direction selectivity but, at the lowest temperature, the response to movement in either direction was often very weak.

5. Velocity Selectivity

Velocity tuning curves were prepared for 8 neurons in area 21a. No systematic variation in the shape of the tuning curve was found to accompany the cooling of the primary visual cortex. In 4 neurons, cooling selectively reduced the response to

FIGURE 6.11

Effect of area 17 temperature on the direction selectivity of 58 area 21a neurons. The solid line (seventh order quadratic best fit curve) and the dotted lines, the 95% confidence intervals, show the tendency for the original direction selectivity to be preserved at temperatures above 10°C and to increase it at lower temperatures. Scattered points at the temperature of 35.5°C, recorded after re-warming of the primary visual cortex, provide a control for the scatter during cooling.



slowly moving stimuli; in 3 others it reduced the responses to faster moving stimuli and in the other, the responses were all uniformly diminished. It was concluded that the effects due to cooling could not be correlated with any particular range of stimulus velocities.

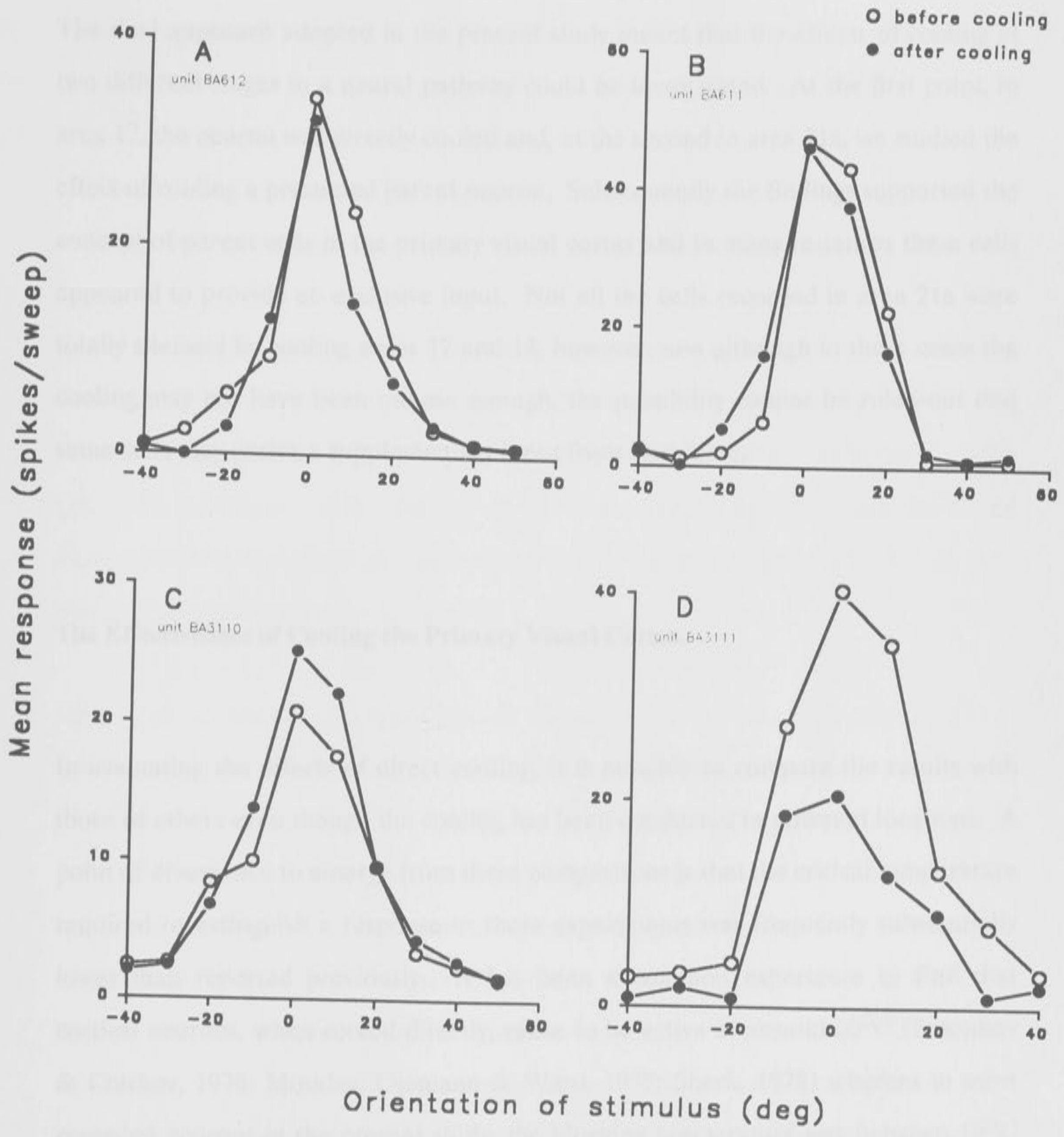
6. Recovery

In 47 of the area 21a neurons responsiveness was tested after re-warming the primary visual cortex to 35.5°C. In 40 cases some recovery was immediately apparent; in 19 of these, the initial recovery was total (from 90% to 120% of the original response level) and only in 7 was it below 40%. In the neurons where the initial recovery was poor or absent, all but one had recovered responsiveness within half an hour of restoring physiological temperature to the primary visual cortex.

The recovery of responsiveness is also demonstrated in Fig.6.12 which shows four examples of tuning curves obtained before cooling and after rewarming of the primary visual cortex. The cooling regime called for the preparation of orientation tuning curves at a number of cooling stages (between 3 and 11) and the subsequent rewarming of the primary visual cortex. In neurons A and B the before and after tuning curves were of an identical height. For neuron C, the responses were higher after the test session whereas, for D, they were substantially lower. In each case, the preferred orientation and its width at half height were unchanged and in most instances there was also little change in responsiveness.

FIGURE 6.12

Orientation tuning curves of four area 21a neurons recorded before cooling and after re-warming of the primary visual cortex to show the level of recovery from cooling. The activity of all these neurons was strongly reduced throughout the cooling phase of the experiment.



DISCUSSION

The dual approach adopted in the present study meant that the effects of cooling at two different stages in a neural pathway could be investigated. At the first point, in area 17, the neuron was directly cooled and, at the second in area 21a, we studied the effect of cooling a presumed parent neuron. Subsequently the findings supported the concept of parent cells in the primary visual cortex and in many instances these cells appeared to provide an exclusive input. Not all the cells recorded in area 21a were totally silenced by cooling areas 17 and 18, however, and although in these cases the cooling may not have been intense enough, the possibility cannot be ruled-out that some cells may derive a supplementary input from elsewhere.

The Effectiveness of Cooling the Primary Visual Cortex.

In evaluating the effects of direct cooling, it is possible to compare the results with those of others even though the cooling has been conducted in different locations. A point of divergence to emerge from these comparisons is that the critical temperature required to extinguish a response in these experiments was frequently substantially lower than reported previously. It has been a common experience to find that cortical neurons, when cooled directly, cease to be active at around 20°C (Sosenkov & Chirkov, 1970; Moseley, Ojemann & Ward, 1972; Sherk, 1978) whereas in most recorded neurons in the present study, the blocking temperature was between 10°C and 20°C and in a small proportion it was even less than 10°C. A potential source for this discrepancy could be the use, in earlier experiments, of large thermistors encased in metal needles which, through their own conductance, could raise the

temperature of the sensor. In the present experiment micro-thermocouples built with 25mm thick wires were used. I was not alone with my findings, however, and other experimenters have also reported low blocking temperatures when small thermocouples were used (50mm wires; Girard & Bullier, 1989) in the monkey cortex. The blocking temperatures in this study were as low as 4°C and the most common blocking temperature was between 10°C and 18°C. Blocking temperatures as low as 5°C to 10°C have also been reported in an experiment on cultured neurons, where the size of the thermosensor should be less critical (Gahwiler, et al., 1972).

Lower blocking temperatures in the present study are also consistent with the finding that the EEG and evoked potentials were present at temperatures above 10°C (Jasper, et al., 1970; Kalil & Chase, 1970; Benita & Conde, 1972; Schiller et al., 1974; Schmielau & Singer, 1977). Since both EEG and evoked potentials are developed from sizeable populations of neurons it would seem that many cells would have blocking temperatures lower than 10°C.

During an experiment it was difficult to estimate the exact spatial extent of deactivated cortex attributable to cooling but it was important to have some idea of the spread for two reasons. Firstly, the cooling should extend far enough to deactivate parent cells in area 17 but not so far that it directly diminished the responses of cells in area 21a. From the present data and that of others (Sosenkov & Chirkov, 1970; Gahwiler et al., 1972; Moseley et al., 1972; Sherk, 1978; Girard & Bullier, 1989) it is possible to infer that the majority of cortical neurons will have declined to about a third of their original responsiveness at a temperature of 20°C and that the depth-temperature gradient, measured directly in the present experiments, would be close to 5°C/mm (see Girard & Bullier, 1989, for comparable findings). Based on these figures the cortex would reach a temperature of 20°C at a depth of 4mm when the full cooling power produced temperatures of

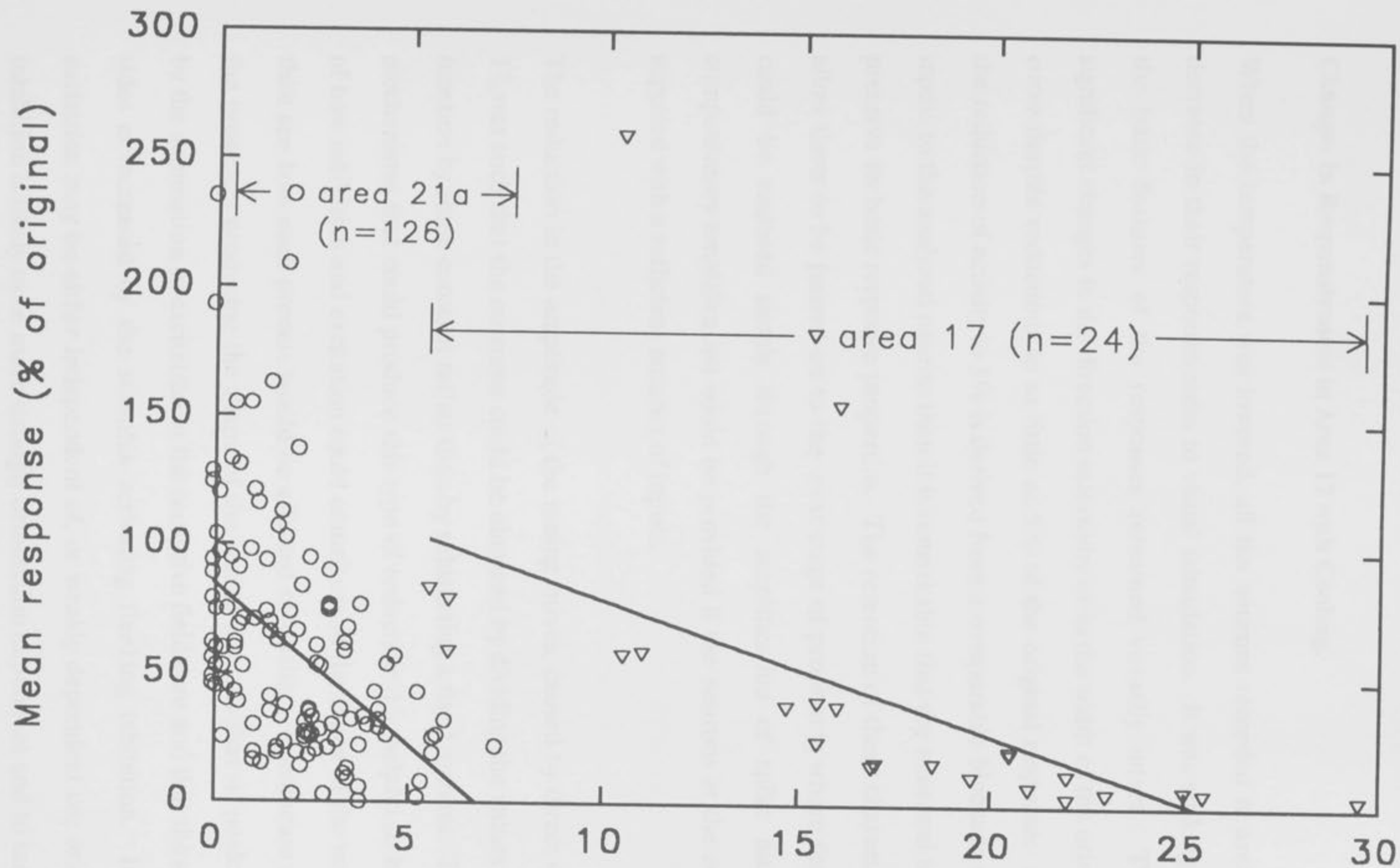
5°C at a depth of 1mm. When compared with the visual field maps (Bilge et al.,1967; Tusa et al.,1978,1979) the extent of cooled cortex (at less than 20°C) corresponded to the area representing the central 5 deg of the visual field in both areas 17 and 18. In contrast, the central representation in area 19, at the same antero-posterior co-ordinates as the cooling probe, is located on the lateral wall of the lateral sulcus, where the temperature is likely to be above 20°C.

The second requirement, that excessive cooling should not spread into the recording region in area 21a, seemed to be met since electrode tracks in the present study were located at a lateral distance of 7-8mm from the edge of the cooling plate. In addition, the lateral sulcus separates the primary visual cortex from the suprasylvian gyrus, the location of area 21a. In monkey cortex, where the temperature gradients are similar, it has been possible to record responses from cells 3mm beyond the cooling plate (Schiller & Malpelli, 1977; Girard & Bullier, 1989).

Since the temperature fall was measured directly in both cortical areas in the present experiment, it is possible to assess the effect coming from the spread of cold into area 21a. To complete the analysis, however, it was necessary to assume that the neurons in areas 17 and 21a react in a similar manner at low temperatures. In applying this analogy, the decline in responsiveness for area 21a cells was always more than 40% greater than that anticipated from the effect of direct cooling in area 17. This distinction between the effects of direct and indirect cooling is further emphasised in Fig.6.13, where the reductions of responsiveness in area 21a and area 17 are related to changes in local temperature, resulting from direct cooling in area 17 and from its spread in area 21a. The clustering of the two groups of points and their distinctive regression lines suggest that the changes in activity of area 21a neurons can not be attributed in full to the spread of cooling.

FIGURE 6.13

Superimposed graphs showing the responsiveness (1) of area 17 neurons as a function of the measured temperature fall in area 17 and (2) of area 21a neurons as a function of the corresponding fall in area 21a. The separation of the two sets of data points and the variation in the slope of the two regression lines highlight the difference in the two populations and indicate that the spread of cold from the primary visual cortex could not account for the reduction in response in area 21a.



Temp. fall (°C) in given area produced by cooling area 17

Changes in Responsiveness in Area 17 with Cooling.

When the temperature was lowered, all the neurons recorded in area 17 showed a decrease in their responsiveness to visual stimulation. It was striking however, that the basic features of the responses remained virtually intact. There were no significant changes in the direction selectivity or in the width of the orientation tuning curve despite reductions to as little as 5% of the original response. Assuming that the reduction of activity to 5% is derived from a comparable blockade of the synaptic inputs to the analyzed neuron then it is remarkable that the neuronal network should preserve its basic response properties. The retention of these characteristics would allow them to be passed on to the next stage of processing where the full response could be restored simply through the amplification of spike numbers. Such compensatory amplification could be provided if the neurons at the next stage were supplied with a sufficient number of inputs.

The reduction in the amplitude of the tuning curves, caused by direct cooling in area 17, was such that the outcome could be obtained by dividing the values of the original function by some constant rather than by subtracting a fixed amount. To arrive at the mechanisms that could produce this type of reduction it is helpful to have some idea of how inhibition and excitation could contribute to the shape of the tuning curve and then see how each process would be affected by cooling. A common explanation of the processes producing the shape of the tuning curve is that its peak is determined by the summation of excitation in the receptive field core and that the steepness of its sides is increased by the stimulus activating flanking inhibition. In this scheme excitation may be either independent of, or weakly dependent on, orientation while inhibition is likely to be more strongly orientation dependent and to increase as more

of the flanking region is activated by the orientations more removed from the peak of the tuning curve.

If we assume symmetry in the tuning curve, then its shape can be defined by two attributes, its peak height and its width. A modification of either of these two features with cooling can also be interpreted, in terms of the scheme above, as a change in excitation and/or inhibition. A decline in peak height would point to a reduction in the excitatory input and if, in addition, there was a reduction in width it would suggest that inhibition was less severely restricted, in relation, to excitation. If cooling reduces excitation and inhibition, each in fixed proportion as through division, then the resultant tuning curve will diminish but will maintain a constant width. A subtractive reduction of one of the inputs with cooling, irrespective of the behaviour of the other, would result in an alteration of the width of the tuning curve. The finding that cooling diminishes the tuning curve in a divisive manner by reducing the peak height without changing its width, therefore, indicates that both excitation and inhibition are similarly diminished in fixed proportion.

The concept that cooling removes inhibition by division is in keeping with earlier proposals that orientation specificity is mediated through divisive inhibition (Rose, 1977; Morrone, et al, 1982; Martin, 1985). In addition, an anatomical substrate appears to be present for divisive inhibition (Blomfield, 1974) in the form of inhibitory terminals located at the cell soma. Some counter evidence appears to exist for the occurrence of divisive inhibition, however, in that a recent study of area 17 cells by Douglas & Martin (1991), failed to detect equivalent changes in membrane potential required by soma-initiated inhibition. The findings of the present study would suggest that it is worthwhile to continue the search for a cellular mechanism for divisive inhibition.

Responsiveness in Area 21a Following Cooling of Area 17.

If the decline in responsiveness in area 21a is attributable, only in small degree, to the direct spread of cooling then the deactivation of the cells in the primary visual cortex can be held responsible. The cells in area 21a, that were completely silenced by cooling the primary visual cortex, should be regarded as true daughter cells and their numbers are sufficient to justify treating area 21a as the second stage of a serial sequence. With the prospect of a population of exclusively dependent daughter cells, area 21a differs from area 18 which, in the cat, works to a large extent in parallel with area 17 (Dreher & Cottee, 1975; Sherk 1978 -but see Donaldson & Nash 1975). Although the results demonstrate the functional dependence that area 21a has upon areas 17 and 18 they do not provide an insight into the course taken by the linking pathway (Symonds et al., 1981; Symonds & Rosenquist, 1984a,b; Rosenquist, 1985; Dreher, 1986; Sherk, 1986). This path could flow directly through cortico-cortical connections or possibly indirectly via the cortico-recipient zone of the thalamus (Graybiel & Berson, 1981a,b; Berson & Graybiel, 1983; Dreher, 1986). Determining which of these is the prime contributor will call for a more sophisticated application of the cooling technique than that employed in the present experiment.

The finding, presented in Fig.6.8, that the cooling of the primary visual cortex extinguished the response in many but not all neurons in area 21a raised the prospect of a supplementary input to a group of neurons in area 21a. This absence of total blockade could be attributed to a failure to cool all the afferent neurons projecting to area 21a but other findings were difficult to explain in terms of a pure sequential relationship between areas 17 and 21a. One of these difficulties is to be found in the result that mild cooling of the primary visual cortex caused an increase in responsiveness in 20% of neurons in area 21a but in none of the neurons of area 17 .

There are a number of earlier reports that spinal and cerebral neurons become hyper-responsive when cooled only 1°C - 2°C below physiological temperature, presumably because of increased resistance of the post-synaptic membrane (for reviews see Brooks, 1983). Such small temperature drops could spread into area 21a if the temperature of the primary visual cortex was reduced below 20°C but the increased responsiveness in area 21a was invariably noticed at much higher temperatures. On the other hand, the presence of increased responsiveness is unlikely to have been overlooked in area 17 since it has been reported to occur over a 10°C range (Brooks, 1983) and tested neurons in area 17 were cooled in steps of 5°C .

Another possible explanation for the increase in responsiveness could be the depolarization of presynaptic terminals as reported in spinal and cuneate neurons (Brooks, 1983). This depolarization occurs within the temperature range of 30°C - 34°C , which is generally below the level of spreading coldness into area 21a, and is more common in the direct cooling of area 17 where there was no sign of an increased responsiveness.

It is possible that, in some instances, a spread of cooling to area 21a caused depolarisation to occur but this would have eventuated at only very low temperatures in the primary visual cortex. Results demonstrate, however, that the sharp increase in activity which characterised some area 21a cells during the early stages of cooling occurred, in many instances, when little or no fall in temperature in area 21a was evident. This suggests that the burst of activity resulted from depolarisation of directly cooled cells in the primary visual. In addition, if the activity of cells in area 21a was largely attributable to a spread of cooling from the primary visual cortex, then the correlation co-efficient which relates area 17 temperature to neuronal activity in area 17, would have been similar to the co-efficient which relates area 17

temperature to area 21a neuronal activity. The relevant correlation co-efficients (r), however, are -0.7 and -0.3, respectively.

The major conclusion reached by this investigation is that many of the cells of area

In the present investigation failure to find an increased responsiveness in area 17 that corresponded to the response enhancement in area 21a lead to a search to explain its appearance in area 21a. Unfortunately, at this stage, most of the possibilities have unsatisfactory features that require further evaluation. For example, it is possible that the area 17 neurons sending axons to area 21a are uniquely affected by mild cooling but have gone unencountered in the present study. Another possibility is that there is an inhibitory pathway, arising from the primary visual cortex, that is more susceptible to cooling than the excitatory one. Such a selective influence on inhibition is not reflected, however, in the orientation tuning curves, direction selectivity or spontaneous activity of area 21a neurons.

Despite the difficulties in explaining all the details of the changes in responsiveness with cooling there remain good reasons for believing that area 21a is principally dependent on the primary visual cortex for its input. For example, there is strong support in the finding that response properties of neurons in area 21a (such as cut-off velocity, orientation tuning and composite ON/OFF responses to flashing stimuli; Wimbome & Henry, 1992) can all be simply derived from C or complex cells of area 17. In these terms, if the cooling of area 17 blocked the input to area 21a it would remove the C cell drive. The neurons of area 21a that are only partially extinguished by cooling the primary visual cortex presumably receive input from neurons other than those with a C type receptive field. This secondary input should be reflected in the receptive field properties of this group of neurons and in future investigations it would be worthwhile to look for a distinctive receptive field in this type of neuron.

Conclusion

The major conclusion reached by this investigation is that many of the cells of area 21a receive their input exclusively from the primary visual cortex. Other cells which continued to respond despite extreme reduction of temperatures in the primary visual cortex, may receive additional signals from elsewhere.

Aims and Objectives

Area 21a is one of many regions of the cat's visual cortex which contains a representation of the visual field and, although there has been some physiological and anatomical research, **CHAPTER 7** area, there is little detailed knowledge of its specific function and pattern of connectivity. The present study was undertaken in order to remedy this situation and my initial aim was to achieve two ends.

SUMMARY AND OVERVIEW

The first purpose of the study was to assess the response characteristics of area 21a in visual perception. By assessing the response characteristics of the area's cells to an array of visual stimuli, it was hoped to determine the cells' particular role, to demonstrate whether or not they comprise a single, unique functional group and to discover, therefore, whether the area represents a region of functional specialisation.

The second purpose was to map the area's pattern of thalamic and cortical afferent connections. Connectivity maps are indicative of the existence of parallel and serial pathways by which a cortical area receives visual signals and, in the case of area 21a, they provided a clue to the level occupied by the area in the sequence of visual processing. Particular note was taken of the strength of neuronal connections between areas with a view to assessing their importance. In the case of the cortex, an investigation of the laminar distribution of cells projecting to area 21a provided an insight into the pattern of neuronal circuitry which underpins the cells' responses.

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of area 21a in considerable detail, the investigation also pin-pointed the primary source of the cells' inputs, postulated a classification of the cells from which the input arose, suggested a position for area 21a in the visual hierarchy and proposed a pattern of information flows which could be the basis of a model for the entire cortex. These findings are summarised in the following sections.

The experiments in which the primary visual cortex was progressively deactivated by cooling provided a new insight into the way in which excitation and inhibition are suppressed and how area 21a is serially dependent on the deactivated region.

Area 21a receives both intracortical (supragranular and infragranular) signals from the primary visual cortex and comparatively minor input from Area 17. In addition, feedback (supragranular and/or infragranular) signals to area 21a arise from areas 17 and 18.

Connectivity and the Place of Area 21a in the Visual Hierarchy

(1) Thalamic Connectivity

Axonal tracer experiments indicate that area 21a is the recipient of both transcortical (serial) and transthalamic (parallel) pathways. Afferent projections arising from the LGNd and pulvinar (tectorecipient zone), however, are relatively weak and may not carry substantial retinal information. Only projections from the LPI (cortico recipient zone) seem robust enough to provide a major thalamic input to area 21a. However, since the LPI receives its inputs from areas of the cortex its projections to area 21a are not considered to constitute a legitimate secondary or parallel pathway.

Experiments which involved extracellular recording combined with controlled

The finding that cells projecting from the LPI to area 21a and to the striate cortex arise from a region that stains weakly for ACh.E lead to experiments which showed that both populations of cells are located conjointly in the LPI. This result adds support to the possibility that information passes between areas 17

and 21a via the LPI and that the LPI resides at the midpoint of a bidirectional cortical loop. Other studies suggest that area 21a may be conforming to a general pattern in which cortical activity in most visual areas is referred back to the LPI.

(2) Cortical Connectivity

At the cortical level the tracer studies indicate that area 21a is reciprocally connected with areas 7, 17, 18, 19, 20a, 20b, 21b, PMLS, PLLS, DLS, VLS and PS. Area 21a receives major feedforward (supragranular laminae) signals from the primary visual cortex and comparatively minor ones from area 19. In addition, feedback (infragranular laminae) signals to area 21a arise from area 19 and from areas of the association cortex listed above.

The relative strengths of supragranular and infragranular connections between specific areas might provide a clue to the place in the hierarchy of visual processing held by those areas. Thus areas 17 and 18 which provide strong supragranular projections appear to occupy jointly, a lower level in the hierarchy than area 21a. Since area 19 has supragranular and infragranular connections with area 21a of about equal strength, it occupies a different position in the hierarchy than areas 17 and 18. Areas of the association cortex which send infragranular projections to area 21a are believed to lie at higher levels.

Experiments which involved extracellular recording combined with controlled cooling of the primary visual cortex also shed light on the origin of inputs to area 21a. The phenomenon by which the responsiveness of some area 21a cells could be totally blocked while others remained active (albeit at a reduced level) suggested that area 21a contains two populations of cells. One of these

populations is fed exclusively by precursors in the primary visual cortex; the other population also receives inputs from the primary visual cortex and in addition might receive signals from elsewhere.

An investigation into the effect which destruction of the primary visual cortex by cooling has on the response properties of area 21a cells revealed the basic response features such as direction selectivity and sharpness of orientation tuning.

The Response Characteristics of Area 21a Neurons

Since the interaction of excitatory and inhibitory inputs are thought

Extracellular recording from the cells of area 21a generally confirmed the findings of earlier investigators who found that the cells had moderately sized receptive fields which were centrally located, were binocular, orientation selective and responded preferentially to slow moving stimuli. A particularly surprising result, especially in the light of the cells' acute sharpness in orientation tuning which often exceeded that of S cells of the striate cortex, was an absence of inhibition in the receptive fields. Consistent similarities in the cells' responses suggested that they comprise a single homogeneous class and, with the exception of acuteness orientation tuning, their responses resembled those of C-type cells of lamina 3 of the striate cortex. The sharper tuning for orientation was explained merely by the presence of a threshold barrier and the response characteristics displayed by the cells added little to those reported in the cells of area 17. This finding argues against the proposition of functional specialisation in area 21a and favours the proposal that the processing step to area 21a simply improves the signal/noise ratio of a response.

A Possible Role for Area 21a

The finding that all area 21a cells are binocular and many are acutely tuned for stimulus orientation indicated possible roles in the detection of spatial disparity and/or orientation disparity. These possibilities were investigated in a separate

visual cortex (principally the striate cortex) either directly or via the LPI (control-

series of experiments but there is no evidence that the cells were selectively tuned for orientation disparity.

An investigation into the effect which deactivation of the primary visual cortex by cooling has on the response properties of area 21a cells revealed that basic response features such as direction selectivity and sharpness of orientation tuning are retained almost to the point where the cell's response is completely extinguished. Since the interaction of excitatory and inhibitory inputs are thought responsible for orientation sharpness and direction selectivity, this finding indicates that the deactivation of excitatory and inhibitory inputs which synapse in area 21a was occurring at the same rate. The dependence of most of area 21a on an input from the primary visual cortex confirmed the existence of a direct serial link but the partial resistance of a group of area 21a cells to deactivation suggested there may be a small supplementary afferent supply.

Is There a Primate Homologue of Area 21a?

Based on a detailed analysis of the cells' responses, especially those related to receptive field location, orientation specificity and preference for stationary/slow-moving stimuli, it is concluded that the cells operate as pattern detectors - being particularly concerned in the analysis of contours of quite specific orientation located in the central part of the visual field.

A Possible Role for Area 21a

In a final appraisal, the axonal tracer studies and deactivation experiments indicate that the major part of the input to area 21a comes from the primary visual cortex (particularly the striate cortex) either directly or via the LPI (cortico-

recipient zone) of the thalamus. This relationship, together with the finding that many area 21a cells are more sharply tuned for orientation than the cells of area 17, raise the possibility that one role of area 21a is **to refine orientation tuning inputs derived from parent cells**. The feasibility of this hypothesis was demonstrated with the use of a model which assumes the presence of a threshold barrier in area 21a that blocks low level incoming firing rates from area 17. Under this model orientation tuning curves derived from C-type cells of lamina 3 in area 17 could be sharpened in area 21a. If this is correct, the result suggests that area 21a has a specialised role in the modification of visual signals which it receives almost exclusively from the primary visual cortex although a small proportion of cells receive a supplementary input from elsewhere, possibly from area 19.

Is There a Primate Homologue of Area 21a?

The possibility that there exists within the primate brain a homologous region to cat area 21a has been supported by several studies of the ventral posterior (VP) extrastriate cortical area of both the macaque and owl monkeys (Newsome and Allman, 1980; Newsome, Maunsell and Van Essen, 1986; Burkhalter and Van Essen, 1986; Burkhalter, Felleman, Newsome and Van Essen, 1986). Topographically, both area 21a of the cat and area VP of the primate are separated from the primary visual cortex by one visual area - area 19 in the case of cats and area V-2 in primates. More striking, however, are similarities in the receptive field characteristics of the cells of both regions. For instance, in areas VP and 21a the central portion of the contralateral visual hemifield is strongly represented. Most neurons in both areas are not highly directional selective but they are selective for orientation (although there is a higher proportion of non-

orientation selective cells in area VP than in area 21a). Virtually all cells in both areas are tuned for velocity and show a distinct preference for slow moving stimuli. Finally, a large proportion of neurons in areas 21a and VP are binocular and few exhibit the hypercomplex property of end-stopping.

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 15 If area VP is a homology of area 21a, it would imply that the carnivore and
 20 primate lines separated from a common ancestral line after areas VP and 21a had
 25 evolved (Kaas, 1980; Baker, Peterson, Newsome and Allman, 1981). On the
 30 other hand, if the areas comprise analogues, the similarities between them
 35 suggest that areas organised in such a fashion represent an obligatory step in a
 40 visual processing hierarchy (see Van Essen, 1985; Rosenquist, 1985; Dreher,
 45 1986; Kaas, 1989; Kaas and Krubitzer, 1991).

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